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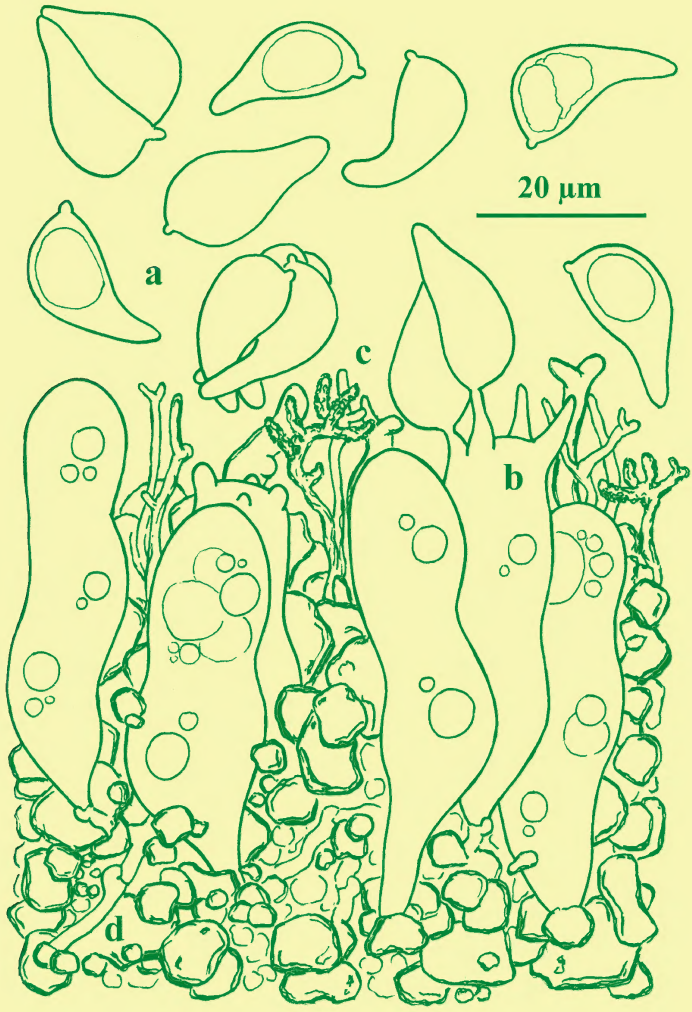

Dendrothele latenavicularis sp. nov.
(Gorjón, Greslebin & Rajchenberg— PLATE 2, p. 104)
SERGIO P. GORJÓN, *artist*

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VOLUME ONE HUNDRED SEVENTEEN — TABLE OF CONTENTS

Cover section

Reviewers vii
Submission procedures viii
From the Editor ix

Research articles

Cortinarius callimorphus, a new species from northern California
Dimitar Bojantchev & R. Michael Davis 1

A new *Phylloporus* from two relict *Fagus grandifolia* var. *mexicana* populations in a montane cloud forest
Leticia Montoya & Victor M. Bandala 9

Type studies on two species of *Phylloporus* (*Boletaceae, Boletales*) described from southwestern China
Nian-Kai Zeng, Li-Ping Tang & Zhu L. Yang 19

Arthrobotrys latispora, a new nematode-trapping fungus from southwest China
Hongyan Su, Shuoran Liu, Yunxia Li, Yong Hong Cao, Minghui Chen & Xiaoyan Yang 29

Additional records of *Volvariella dunensis* (*Basidiomycota*, *Agaricales*): morphological and molecular characterization
Alfredo Vizzini, Marco Contu & Alfredo Justo 37

Moelleriella pumatensis, a new entomogenous species from Vietnam
Suchada Mongkolsamrit, Tai Toan Nguyen, Ngoc Lan Tran & J. Jennifer Luangsa-ard 45

Lepiota (Agaricales) in northern Thailand – 1. *Lepiota* section *Stenosporae*
P. Sysouphanthong, K.D. Hyde, E. Chukeatirote, A.H. Bahkali & E.C. Vellinga 53

New records of *Digitoramispora* from China
Yi-Dong Zhang, Jian Ma, Li-Guo Ma & Xiu-Guo Zhang 87

Seven new records of foliicolous lichens from Vietnam
Thi Thuy Nguyen, Yogesh Joshi, Robert Lücking, Anh Dzung Nguyen, Xin Yu Wang, Young Jin Koh & Jae-Seoun Hur 93

Dendrothele latenavicularis sp. nov. (*Agaricales, Basidiomycota*) from the Patagonian Andes
Sergio P. Gorjón, Alina G. Greslebin & Mario Rajchenberg 101

Endocalyx melanoxanthus var. *melanoxanthus* (*Ascomycota*): new to Brazil and three new hosts
Nadja Santos Vitoria, Maria Auxiliadora Q. Cavalcanti, Edna Dora Martins Newman Luz & José Luiz Bezerra 109

New records of rust fungi on sedges (*Cyperaceae*) from Pakistan
Malka Saba & Abdul Nasir Khalid 115

A new species and new records of *Endophragmiella* from China
Shou-Cai Ren, Jian Ma & Xiu-Guo Zhang 123

Two new *Minimelanolocus* species from southern China
Jian Ma, Yi-Dong Zhang, Li-Guo Ma & Xiu-Guo Zhang 131

Two new species of *Passalora* and *Pseudocercospora* from northeastern Uttar Pradesh, India
Raghvendra Singh, Shambhu Kumar & Kamal 137

Lecanora wrightiana and *Rhizocarpon inimicum,* rare lichens new to Turkey and the Middle East Kenan Yazici, Andre Aptroot & Ali Aslan 145

A new volvate *Macrolepiota* (*Agaricomycetes, Agaricales*) from Italy, with observations on the *M. procera* complex Alfredo Vizzini, Marco Contu, Stefano Ghignone & Else Vellinga 149

Studies in *Amanita* (*Amanitaceae*) of Central America. I. Three new species from Costa Rica and Honduras R.E. Tulloss, R.E. Halling & G.M. Mueller 165

Studies on *Cephalotrichum* from soils in China —twelve new species and two new combinations Yu-Lan Jiang, Jun-Jie Xu, Yue-Ming Wu, Yue-Li Zhang, Hui-Mei Liu, Hao-Qin Pan & Tian-Yu Zhang 207

Morphology and phylogeny of *Pseudocercospora kamalii* sp. nov., a foliar pathogen on *Terminalia* from India Kunhiraman C. Rajeshkumar, Rahul Sharma, Rahul P. Hepat, Santosh V. Swami, Paras Nath Singh & Sanjay K. Singh 227

Mycena sect. *Longisetae*: a new species, a new name, and an addition Dollymol M. Aravindakshan & P. Manimohan 239

Three new hyphomycetes from southern China Jian Ma, Li-Guo Ma, Yi-Dong Zhang & Xiu-Guo Zhang 247

A new species of *Junghuhnia* (*Basidiomycota, Meruliaceae*) from tropical China Hai-Sheng Yuan 255

Caloplaca allochroa (lichenized *Ascomycetes*), a new saxicolous lichen species from South Korea Yogesh Joshi, Jan Vondrak, Olga Vondráková, Thi Thuy Nguyen & Jae-Seoun Hur 261

Four anamorphic fungi (with two new species) from forests of Western Ghats, India J. Pratibha, D.J. Bhat & S. Raghukumar 269

Taxonomic studies of *Endophragmiella* from southern China Li-Guo Ma, Jian Ma, Yi-Dong Zhang & Xiu-Guo Zhang 279

Rhexodenticula zhengii sp. nov. from fallen leaves from China De-Wei Li, Jingyuan Chen & Yixun Wang 287

Septobasidium atalantiae sp. nov. (*Septobasidiaceae*) and *S. henningsii* new to China Suzhen Chen & Lin Guo 291

Revision of *Glomeromycetes* with entrophosporoid and glomoid spore formation with three new genera Fritz Oehl, Gladstone Alves da Silva, Iván Sánchez-Castro, Bruno Tomio Goto, Leonor Costa Maia, Helder Elísio Evangelista Vieira, José-Miguel Barea, Ewald Sieverding & Javier Palenzuela 297

Puccinia cortusae (*Basidiomycota*; *Uredinales*) on *Cortusa brotheri* (*Primulaceae*), new to southern Asia (Fairy Meadows, Pakistan) A.N. Khalid & M. Saba 317

Gymnopus fuscotramus (*Agaricales*), a new species from southern China Armin Mešić, Zdenko Tkalčec, Chun-Ying Deng, Tai-Hui Li, Bruna Pleše & Helena Ćetković 321

Biogeographical patterns in pyrenomycetous fungi and their taxonomy. 2. Additions to the Grayan disjunction Larissa N. Vasilyeva & Steven L. Stephenson 331

Lignincola conchicola from palms with a key to the species of *Lignincola*
J.K. Liu, E.B.G. Jones, E. Chukeatirote, A.H. Bahkali & K.D. Hyde 343
Craspedodidymum and *Corynespora* spp. nov. and a new anamorph recorded from southern China Li-Guo Ma, Jian Ma, Yi-Dong Zhang & Xiu-Guo Zhang 351
A new species of *Lepiota* (*Agaricaceae*) from southwestern China
Jun F. Liang & Zhu L. Yang 359
Passalora wangii comb nov. from the genus *Tandonella*
Feng-Yan Zhai, Ying-Lan Guo, Ying-Jie Liu & Yu Li 365
A new species of *Terriera* (*Rhytismatales*, *Ascomycota*) from China
Zhong-Zhou Yang, Ying-Ren Lin & Cheng-Lin Hou 367
New Bulgarian records of fungi associated with glacial relict plants
Cvetomir M. Denchev, Dimitar Y. Stoykov, Ekaterina F. Sameva & Boris Assyov 373
Two new *Ceratocystis* species associated with mango disease in Brazil
Marelize Van Wyk, Brenda D. Wingfield, Ali O. Al-Adawi, Carlos Rossetto, Margarida Fumiko Ito & Michael J. Wingfield 381
The genus *Cladonia* (lichenized *Ascomycota, Cladoniaceae)* in South Korea
Xin Yu Wang, Yogesh Joshi & Jae-Seoun Hur 405
First record of *Resupinatus poriaeformis* (*Agaricomycetes*) from South America
Georgea Santos Nogueira-Melo, Leif Ryvarden & Tatiana Baptista Gibertoni 423
New recombinations in *Glomeromycota*
Fritz Oehl, Gladstone Alves da Silva, Bruno Tomio Goto & Ewald Sieverding 429
Pouzarella (*Agaricales*, *Entolomataceae*) species from New South Wales (Barrington Tops National Park) and northeastern Queensland, Australia
David L. Largent, Sarah E. Bergemann, Griffin A. Cummings, Kathryn L. Ryan, Sandra E. Abell-Davis & Skye Moore 435
Amanita vernicoccora sp. nov. — the vernal fruiting "coccora" from California
Dimitar Bojantchev, Shaun R. Pennycook & R. Michael Davis 485
Book reviews and notices Else C. Vellinga (Editor) 499
Mycobiotas online
Abstracts of newly posted annotated regional species lists 508
Nomenclature
Changes to publication requirements made at the XVII International Botanical Congress in Melbourne —what does e-publication mean for you?
Sandra Knapp, John McNeill & Nicholas Turland 509
Nomenclatural novelties proposed in Mycotaxon 117 517

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From the Editor-in-Chief

Mycotaxon 117 and beyond— Welcome to yet another (!) late volume. In the previous volume, we noted the delay caused by our attending the ground-breaking 2011 Melbourne International Botanical Congress (see p. 509 for changes needed for new effective electronic publication of names). This was compounded during publication of Mycotaxon 116 when our host service's trainee uploaded Mycotaxon 116 using the wrong algorithm, thereby linking titles to the wrong pdf files, marking Open Access papers as Subscription, and deleting one first author completely from the journal site. Ingentaconnect assures us that this problem will not occur again. We apologize to readers and authors who visited the journal site during Ingenta's frantic September 16–22 adjustment and thank Hai-Jiao Li for being so patient until the final author-listing repair was complete. Fortunately, Mycotaxon 117 offers several groundbreaking monographs, keys, and excellent taxonomic papers that make it well worth the wait.

Although your *Editor-in-Chief* is still scrambling to catch up, our next two volumes are now full and undergoing final editorial review. With our July–September volume finally finished, we anticipate —perhaps unrealistically— that Mycotaxon 118 will appear on the distal side of 'on-schedule.'

The new 'DOI' prefix— CrossRef has changed its standard prefix from 'doi:' to 'http://dx.doi.org/'. Those wishing to link immediately to a cited publication (rather than browse Google) should search the web using the new prefix.

January 1 welcomes the English diagnosis— In 2012, the International Code of Nomenclature for algae, fungi, and plants (ICN) accepts diagnoses and descriptions written in either Latin or English. Mycotaxon uses this opportunity to limit its required diagnoses for all new taxonomic descriptions to five or fewer lines.

A 'diagnosis' (which we prefer be written in English) should cite only the major diagnostic characters that differentiate a proposed new taxon from its closest known relative. For example, the statement, "Differs from *Manuscriptus optimus* by a bright yellow pileus, smaller ellipsoid smooth basidiospores, lack of cheilocystidia, and association with *Picea sitchensis*." is an acceptable diagnosis. Preceded by the taxonomic name and registration number, it should be followed by type and etymology paragraphs. All additional details, including precise dimensions, belong in the extended technical description, 'Additional specimens examined,' and discussion sections.

Diagnoses are particularly useful in that they list only those character(s) that readily distinguish one taxon from others. The longer technical description outlines all characters in detail. Also important are the closing (and less technical) remarks that compare characters among similar (although not necessarily closely related) taxa. By limiting diagnoses to five or fewer lines, Mycotaxon hopes authors will prepare a straightforward three-part type description, with each part —diagnosis, description, remarks— giving a complimentary yet unique insight into the new taxon.

Warm regards, Lorelei Norvell

Mycotaxon *Editor-in-Chief*
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Cortinarius callimorphus, a new species from northern California

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Abstract — Described is *Cortinarius callimorphus*, a new species in subgenus *Phlegmacium* from the coastal woods of northern California, with Sitka spruce to its apparent primary host associate. *Cortinarius callimorphus,* which resembles other members of sect. *Multiformes*, stands out because of its citriform spores. Its closest known relative is *C. polymorphus* from Europe.

Key Words — *Cortinariaceae*, fungal taxonomy, nrITS data

Introduction

The ecosystem of the northernmost coastal region of California (Del Norte, Humboldt, and Mendocino counties) is influenced by high annual precipitation averages (CalFire 2011) that are characteristic of the Pacific Northwest temperate rainforest and which support its dominant conifers —*Picea sitchensis* (Bong) Carrière (Sitka spruce) and *Tsuga heterophylla* (Raf.) Sarg. (western hemlock). The extensive wet forests of the region support a rich fungal diversity. The types of approximately two-thirds of the *Cortinarius* species described from California have been collected in this region (Smith 1939; Ammirati & Smith 1977; Moser & Ammirati 1997, 2000). *Cortinarius callimorphus*, described here, is common under Sitka spruce along the northern Californian coast.

Materials & methods

Methods for morphological studies and DNA extraction, PCR conditions and primers, PCR product clean up, and sequencing are outlined in Bojantchev & Davis (2011). Color codes follow Munsell™ soil color charts (Anonymous 2000). Terminology follows Brandrud et al. (1998). Collections are stored in the private herbarium of the first author or at the University of California herbarium in Berkeley (UC) where noted.

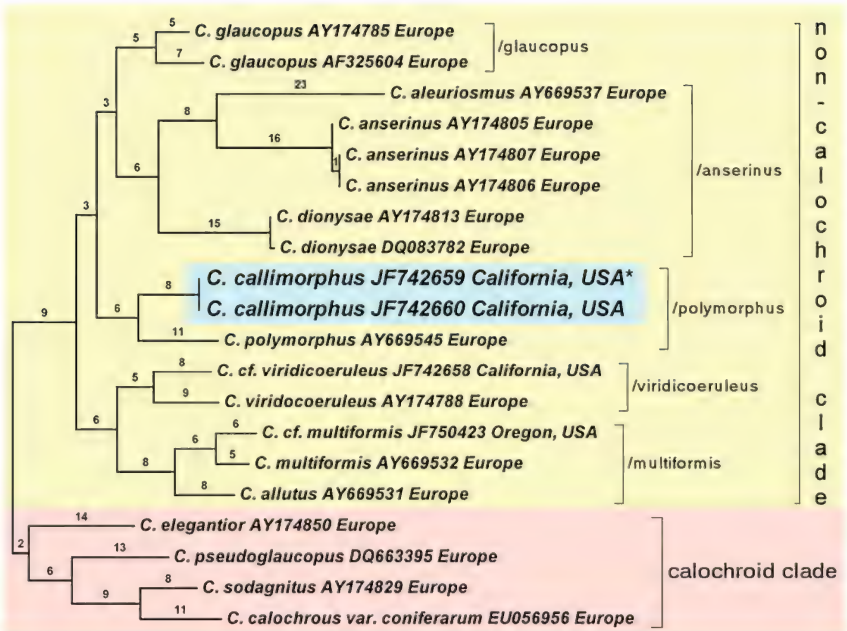

Fig 1. Phylogenetic tree inferred by maximum parsimony analysis of 20 *Cortinarius* subg. *Phlegmacium* nrITS sequences. The tree shows the position of *C. callimorphus* relative to its closest neighboring stirpes and the more distant calochroid clade. Branch lengths represent estimated nucleotide substitutions. The GenBank accession numbers are listed after the taxon names. *Cortinarius calochrous* var. *coniferarum* was selected as outgroup.

Phylogenetic analysis

During our studies we have downloaded and reviewed all *Cortinarius* nrDNA sequences from the public databases GenBank (http://www.ncbi.nlm.nih.gov) and UNITE (http://unite.ut.ee/). Preliminary phylogenetic analysis (not shown) of 984 *Phlegmacium* nrITS sequences from the northern hemisphere, including 278 sequences from our own collections, clarified the closest relatives of *C. callimorphus* and resolved its position outside of the calochroid super-clade as defined by Frøslev et al. (2007) and Garnica et al. (2009).

Twenty *Phlegmacium* taxa were selected for a detailed phylogenetic analysis. Sixteen sequences were sourced from GenBank and four sequences representing western North American taxa came from our collections. The breakdown of the selected sequences is four members of the calochroid super-clade; two sequences of *C. callimorphus* plus one of its closest known relatives; 13 sequences of the most representative taxa of the nearest stirpes to *C. callimorphus* — /anserinus, /viridicoeruleus, /multiformis and /glaucopus from both Europe and Western North America. *Cortinarius calochrous* var. *coniferarum* (M.M. Moser) Nezdojm. was selected as an outgroup.

Sequence alignments were generated with MAFFT v6.821b (Katoh et al. 2002) with the G–INS–i global alignment iterative refinement strategy. Minimal gap opening and

extension penalties were set for better resolution of the more variables sectors within the nrITS. The alignments were visually inspected and corrected where needed.

The evolutionary history was inferred using the Maximum Parsimony method as implemented by MEGA5 (Tamura et al. 2007). The MP trees were generated by the Close-Neighbor-Interchange algorithm with search level 0 in which the initial trees were obtained with the random addition of sequences (10 replicates). The search resulted in nine most parsimonious trees (length = 249) which only differed in the topology of the terminal nodes. One tree was selected (Fig. 1).

Taxonomy

Cortinarius callimorphus Bojantchev & R.M. Davis, **sp. nov.** Figs 2–6

MycoBank MB 561091

Pileo 60–100 mm lato, hemispherico, dein plano-convexo, glutinoso, margine involuto, ochraceoluteo vel flavobrunneo interdum centro, ferrugineo maculato, cum KOH roseobrunneo. Lamellis emarginatis, pallide argillaceis, argillaceobrunneis, in statu senili. Stipite 80–140 mm longo, cylindrico, bulbo submarginato 30–50 mm lato. Velo universale albido. Velo partiale copioso, albido, cum KOH ope nullo. Carne albida. Sapore miti. Sporis 9–10 × 5–6 µm, limoniformibus, verrucosis, basidiis 24–30 × 6–8 µm, tetrasporigeris, fibulis praesentibus.

Type: USA. California: Mendocino County, Caspar, Caspar Cemetery, under *Picea sitchensis*, *22* Nov 2009, Bojantchev DBB25774 (**Holotype** UC 1860824; Genbank nrITS JF742659).

Etymology: from Greek: καλος = beautiful, μορφος = form, shape.

Fig 2. *Cortinarius callimorphus* (collection DBB00080) – a typical form.

straight to involute margin, uniformly ochre yellow (10YR 8/6–8/8), sometimes darker in older basidiomata (10YR 5/6–5/8) with a persistent lighter yellow zone near the margin (2.5Y 8/6) and rusty (5YR 6/6) spots where bruised; surface glutinous when wet, glabrous to dull glossy when dry, innately fibrillose, radially corrugated near the margin at age. Lamellae L = 70–110, crowded, 8–15 mm broad, pale clay (2.5Y 8/1–8/3) to grayish-white when young, never bluish, shading yellow brown to brown (7.5R 6/6–5/6) as the spores mature; edge even; attachment notched; lamellulae abundant. Stipe 80–140 mm long, 10–20 mm wide, cylindrical with a submarginate 30–50 mm wide bulb, white, without bluish tints, spotting brown where bruised. Universal Veil white, leaving volva-like remnants on the bulb. Cortina white, turning rusty brown due to mature spore drop, leaving an annular zone of dense fibrils on the stipe, occasionally forming a hairy appendiculate zone on the pileal margin. Context white, bruising brown. Odor weak, aromatic to none. Taste fungal, not unpleasant. Macrochemical Reactions 5% KOH carmine-red on pileus, uniformly pale yellow brown on context, strongest on mature basidiomata, negative on basal mycelium. UV non-fluorescent. Spore Deposit deep rusty brown.

Basidiospores (8.5–)9.0–10.0(–10.5) × (5.0–)5.2–5.8(–6.0) µm (mean 9.5 × 5.6 µm), Q = 1.54–1.77, Q_{av} = 1.72 (N = 217, 11 basidiomata, seven collections), distinctly citriform, moderately verrucose. Basidia 24–30 × 6–8 µm, 4-spored, cylindro-clavate, clamped. Gill edge fertile or with clusters of elongated septate cells. Cystidia not observed. Pileipellis an ixocutis, duplex; epicutis medium thick, 6–14 layers of hyphae 2–7 µm diam., hyaline, hypodermium well developed, composed of interwoven to parallel cylindrical cells, 20–40 × 12–20 µm with refractive cytoplasmic pigment. Clamp connections common in all parts.

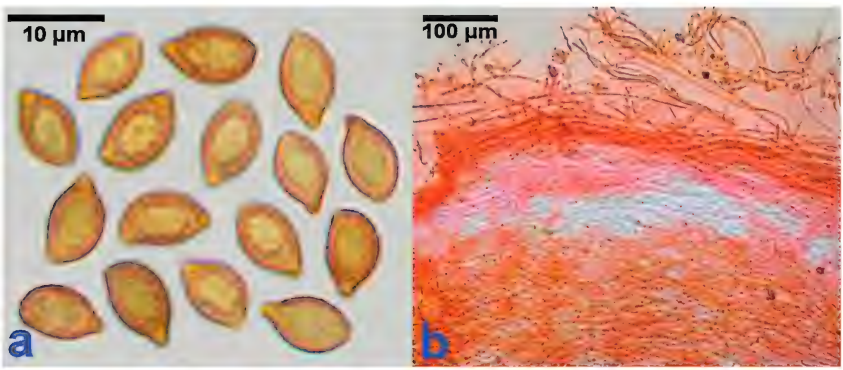

Fig 3. *Cortinarius callimorphus*.
a) Basidiospores (UC 1860824, holotype); b) Cuticle structure (UC 1860824, holotype).

Fig 4. *Cortinarius callimorphus* a) Elongated, septate cells on lamellar edge (UC 1860824, holotype); b) 5% KOH reaction on pileus surface and stipe context (DBB09495); c) Corrugated pileus surface near the margin (DBB09495).

Habitat and distribution — Very common under Sitka spruce in Northern California, particularly in the Mendocino, Humboldt and Del Norte counties. We have never seen it south of Mendocino. Its distribution within the Sitka spruce range is apparently limited to northern California and southern Oregon (where we have collected it once). There are no matching records from Washington and British Columbia despite the intensive collecting and molecular cataloguing that has taken place in these regions. There are no other closely matching North American collections in the public sequence databases.

Fig 5. *Cortinarius callimorphus* collections: a) DBB27800; b) DBB25837.

Fig 6. *Cortinarius callimorphus* collections: a) DBB40200; b) UC 1860824 (holotype)

Additional collections examined: **USA. California:** Mendocino County, Caspar, Caspar Cemetery, under *Picea sitchensis*, 26 Nov 2010, Bojantchev DBB40200; 4 Nov 2007, Bojantchev DBB00080; Jackson State Forest, under *Picea sitchensis*, 22 Nov 2008, Bojantchev DBB09495; Jughandle State Reserve, under *Picea sitchensis*, 22 Nov 2009, Bojantchev DBB25837 (UC 1860825, Genbank nrITS JF742660); Del Norte County, Jedediah Smith Redwood State Park, under *Picea sitchensis*, 7 Nov 2009, Bojantchev DBB22704; Humboldt County, Arcata, HBMS Mushroom Fair, under *Picea sitchensis.* 20 Nov 2010, Bojantchev DBB39217; **Oregon:** Curry County, Samuel Boardman State Park, under *Picea sitchensis,* 11 Nov 2009, Bojantchev DBB27800.

Discussion

Traditional morphological analysis based on the overall combination of stature, a yellowish viscid pileus, and argillaceous lamellae suggests that *C. callimorphus* belongs to sect. *Multiformes* Rob. Henry ex Brandr. & Melot. Its carmine-red KOH reaction on the pileus of and submarginate bulb suggest that *C. callimorphus* might be related to the calochroid cortinarii, but its well-developed hypodermium (duplex pileipellis) supports placement in sect. *Multiformes*.

Within that section, *C. callimorphus* stands apart with its distinctly citriform spores, a relatively uncommon feature in the non-calochroid clades. *Cortinarius polymorphus* Rob. Henry is the only other species known with similar macromorphological features (particularly lacking any blue colors) and similarly shaped citriform spores. Detailed descriptions of *C. polymorphus* are available in Henry (1935, 1951, 1985) and Bidaud et al. (2007); the latter includes quality iconography. Molecular analysis confirms a close relationship between the two species (Fig. 1). *Cortinarius polymorphus* has also been assigned to *Multiformes* by Henry (1935, 1951, 1985), Moser (1960), and Bidaud et al. (2007).

Recent phylogenetic studies within *Cortinarius* (Garnica et al. 2003) and our own nrITS analysis show that sect. *Multiformes* is not well supported. Most taxa traditionally assigned to it belong to various other *Phlegmacium* clades. It is not the aim of this work to present a detailed picture of the infrageneric relationships within subg. *Phlegmacium*, but as shown in Fig. 1, *C. callimorphus* and *C. polymorphus* form a clade that is a sibling to the stirpes around *C. viridicoeruleus* Chevassut & Rob. Henry and *C. anserinus* (Velen.) Rob. Henry. A common character of these clades is the distinctly citriform spores.

Cortinarius callimorphus and *C. polymorphus* are very similar but can be separated based on differences in geography (with the former found only in western North America and the latter in Europe), nrITS sequences, and host associations. *Cortinarius polymorphus* is apparently associated with broadleaved trees, mainly members of *Fagaceae*, while *C. callimorphus* is associated with *Picea* and possibly other conifers. The spores of *C. polymorphus* also appear slightly broader with Q_{av} = 1.5 vs. Q_{av} = 1.7 for *C. callimorphus*. Henry (1935)

describes the lamellar edges of *C. polymorphus* as distinctly denticulate, but we have not observed that feature on the *C. callimorphus* basidiomata.

Complete iconography of *C. callimorphus* and a comparative image study is available on http://www.mushroomhobby.com.

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A new *Phylloporus* from two relict *Fagus grandifolia* var. *mexicana* populations in a montane cloud forest

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Abstract — A new species, *Phylloporus fagicola,* has been discovered growing in association with two relict populations of *Fagus grandifolia* var. *mexicana* in the subtropical region of central Veracruz (eastern Mexico). The species belongs to a taxonomic group characterized by broadly ellipsoid oblong to ovoid basidiospores, distinct from the subfusoid boletoid type. The new taxon is described, illustrated, and compared with closely similar species.

Key words — ectomycorrhizal fungi, neotropical fungi, gilled *Boletales*, taxonomy

Introduction

Currently, *Phylloporus rhodoxanthus* (Schwein.) Bres., *P. bellus* (Massee) Corner, *P. phaeoxanthus* var. *simplex* Singer & L.D. Gómez, *P. foliiporus* (Murrill) Singer, *P. centroamericanus* Singer & L.D. Gómez, and *P. guzmanii* Montoya & Bandala have been recorded from Mexico (Singer 1957, Singer 1978, Singer & Gómez 1984, Montoya et al. 1987, Montoya & Bandala 1991). Those records represent a low proportion of the 24 *Phylloporus* taxa known to occur in the Neotropics (Neves & Halling 2010). A new species described here, *P. guzmanii* from Mexico, and *P. aurantiacus* Halling & G.M. Muell. from Costa Rica (Halling et al. 1999), form a group of American taxa distinguished by broadly ellipsoid, oblong, or ovoid shaped basidiospores that differ from the boletoid (subfusoid) basidiospores typical of other *Phylloporus* species (Corner 1970, Heinemann & Rammeloo 1987, Neves & Halling 2010). The *Phylloporus* species previously reported from Mexico have been recorded growing with *Quercus* or *Pinus*. The new species described here represents the first Mexican record of a *Phylloporus* growing in association with *Fagus grandifolia* var. *mexicana* (Martínez) Little.

Materials & methods

Between September 2005 and September 2009 in the State of Veracruz, random surveys were conducted in two *Fagus grandifolia* var. *mexicana* stands in Mesa de la

Yerba (Acajete; 19°33′37.2″N 97°01′9.8″W, 1900 m) and Acatlán Volcano (Naolinco-Acatlán Road, Acatlán; 19°40′43.9″N 96°51′9.8″W, 1840 m).

Macromorphological characters were recorded from fresh sporocarps. Alphanumeric color codes refer to Kornerup & Wanscher (1967) and Munsell (1994) (e.g. 2.5Y 5/2). Micromorphological features were recorded in 3% potassium hydroxide water solution. Basidiospore measurements were determined according to Montoya & Bandala (2003), with 45–50 spores measured per collection and where X_m = length × width range of means and Q_m = the mean length/width ratio for n collections. Line drawings were made with the aid of a drawing tube. The examined specimens studied are now deposited in XAL herbarium. Acronyms for the herbaria follow Holmgren & Holmgren (1998).

Taxonomy

Phylloporus fagicola Montoya & Bandala, **sp. nov.** FIGS. 1–7

MycoBank MB 519849

Pileus 10–53 mm latus, primo hemisphaerico, dein late convexo vel applanato, sicco, velutinosus, subtomentosus, tenuis squamulosus vel glabro ex parte et ad marginem, aurantioruber, flavus ex parte, cyanescens, cum NH_4 caerulescent. Lamellae decurrentes, confertae, septis connexae, furcatae circa stipitem, crassae, flavidae, rufo maculatae, cyanescens. Stipes 13–40 × 2–7 mm, centralis vel eccentricus, firmus, rufo, aurantioruber, velutinosus, basi villosa. Contextus flavus ad pileus, brunneo-rufo ad stipes, cyanescens, cum NH_4 caerulescent. Basidiosporae 6.5–10.5(–11) × 4–7(–7.5) µm, ellipsoidae, oblongae olivacei-flavae. Pleurocystidia 84–132 × 7.5–13 µm, subcylindrata, subfusiformia, ventricosa, sinuosis, numerosa; pariete tenui vel incrassatus (0.8–3.5µm). Cheilocystidia 26–114 × 4.8–12(–13) µm, clavata, subcylindrata, subfusiformia, interdum mucronata, numerosa; pariete tenui vel incrassatus (–2.5 µm). Pileipellis primo cutis instructa dein intermitto cum numerosis tumulus catenulatis hyphis, cellulae terminales clavatis, subcylindraceis, ventricosis, subfusiformis (12–55 × 3.5–7 µm). Hyphis defibulatis.

Holotypus: *Ad terram in silvis. Mexico. Veracruz, Mpio. Acatlán, Acatlán Volcano, August 21, 2007, del Moral 82 (XAL).*

Pileus 10–53 mm diam., subhemispheric when young, plane-convex to plane, reddish-orange (7C8–B8, 7D6) with some intense bright yellowish tones, yellowish-orange (6B–C8, 10YR 8/8), brick orange to brick-red (2.5YR 4/6–4/8, 3/6), in some areas orange (6B8) and with a yellow ground or towards the margin (3A7, 10 YR 6/8), this latter at times discolouring, bluing to blackening when bruised, dry, pruinose to velutinous, subtomentose to faintly squamulose or subfloccose towards the center, glabrous and smooth in some areas and towards the margin or faintly pruinose at margin, cracked in some areas. Lamellae decurrent, thick, broad, 5–8 mm broad, close to crowded. Lamellulae of 3–5 different lengths, at times furcate towards the stipe attachment, with interparietal veins, joined in "H appearance", egg yellow when young to yellow with orange tinges (5A7–8, 5B7, 6A6–B7) or in age yellow with red (8D8) irregular stains or almost totally red mainly towards the stipe attachment, staining green-blue, bluish-brown (25D6) to grayish-black or blackish, margin

Fig. 1. *Phylloporus fagicola* (Garay 215).
Bar = 10 mm.

irregular. Stipe 13–40 × 2–7 mm, subcylindrical, attenuated towards the base, velutinous to finely furfuraceous towards the apex, central to excentric, dry, firm, apex sulcate by the decurrence of the gills, reddish-orange (8C7), reddish (10C8), vinaceous-brown (7E8) or brick-orange (2.5 4/8), reddish-orange (6B8) at apex, yellow towards the base (4A6–5) but frequently stained dark grayish to blackish; base at times villose, yellowish, with rhizomorphs. Context bright yellow (3A6, 7B7) to egg yellow (4A7–8) at pileus; vinaceous-brown to reddish-orange (7D6–D7) at stipe; staining deep blue (23D7–D8) mainly on stipe area. Odor agreeable, fruity (recalling citrics). Machrochemical reactions: NH4OH stains greyish-black, greenish on pileus; blue, greenish-blue on context. KOH stains yellowish-brown on pileus and lamellae and orange-brown on context.

Basidiospores 6.5–10.5(–11) × 4–7(–7.5) µm, X_m = 7.0–9.0 × 4.2–5.1 µm, Q_m= 1.53–1.78, ellipsoid to oblong, obtuse, yellowish-green, smooth, thin-walled. Basidia 46–60 × 6–7 µm, clavate, subcylindrical, sinuous, attenuated towards the base, bisporic or tetrasporic, sterigma up to 8.5 µm long, with yellow contents. Pleurocystidia 84–132 × 7.5–13 µm, subcylindrical, subfusoid, sinuous, ventricose, frequently thin-walled but other with wall 0.8–3.5µm thick, projected from below the hymenium, abundant, yellow in KOH. Cheilocystidia 26–114 × 5–12(–13) µm, clavate, subcylindrical, mucronate,

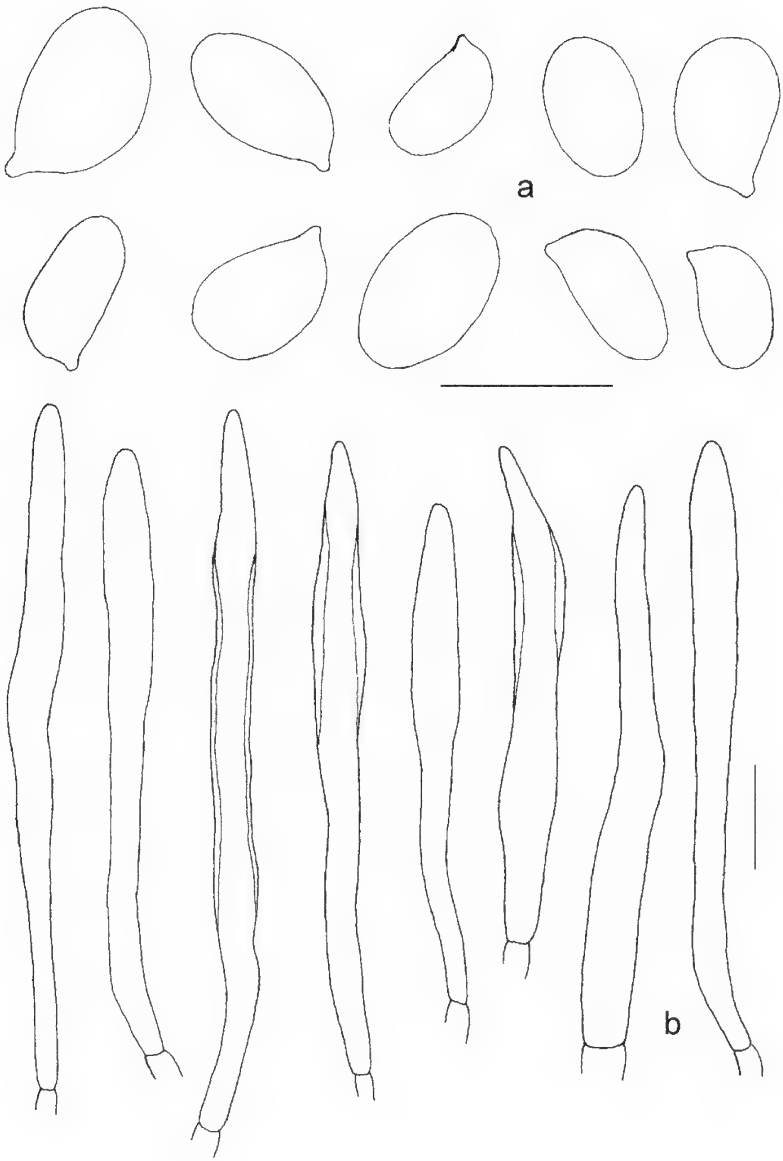

Fig. 2. *Phylloporus fagicola*.
a. Basidiospores (del Moral 117). b. Pleurocystidia (del Moral 82, holotype).
Bars: a = 10 μm, b = 20 μm.

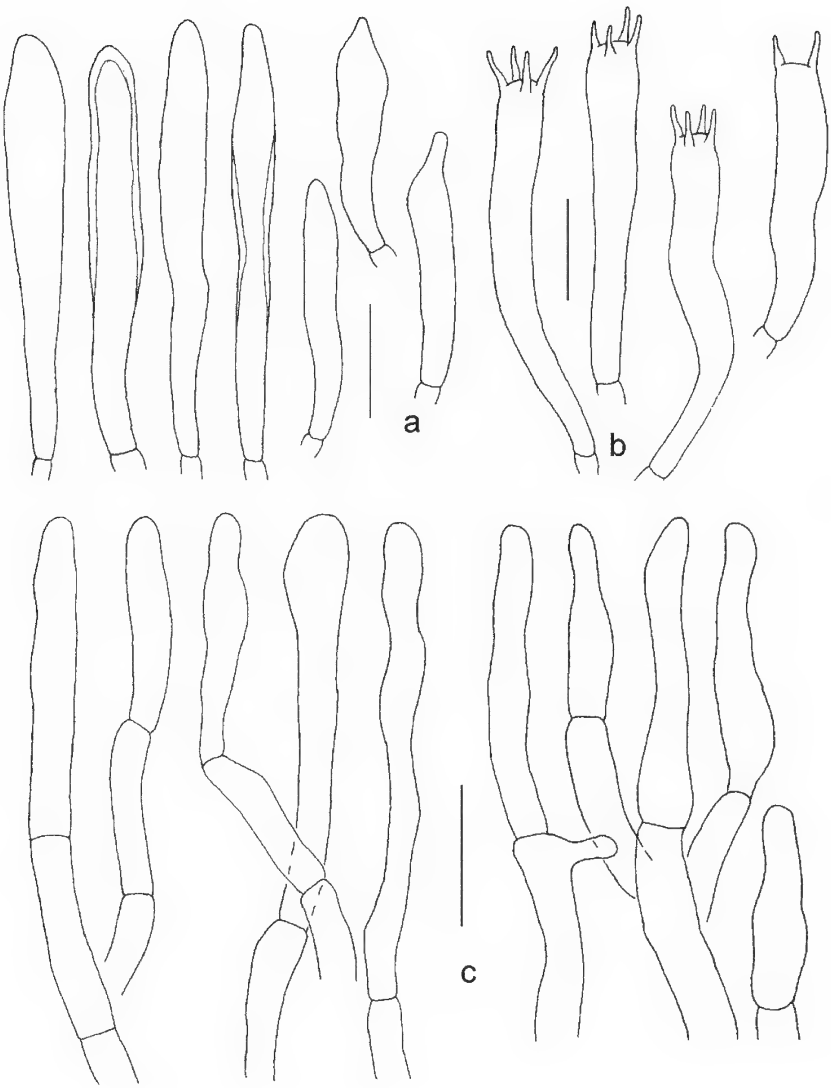

Fig. 3. *Phylloporus fagicola*.
a. Cheilocystidia. b. Basidia. c. Terminal elements of pileipellis.
(a–b = del Moral 82, holotype; c = del Moral 292). Bars = 20 µm.

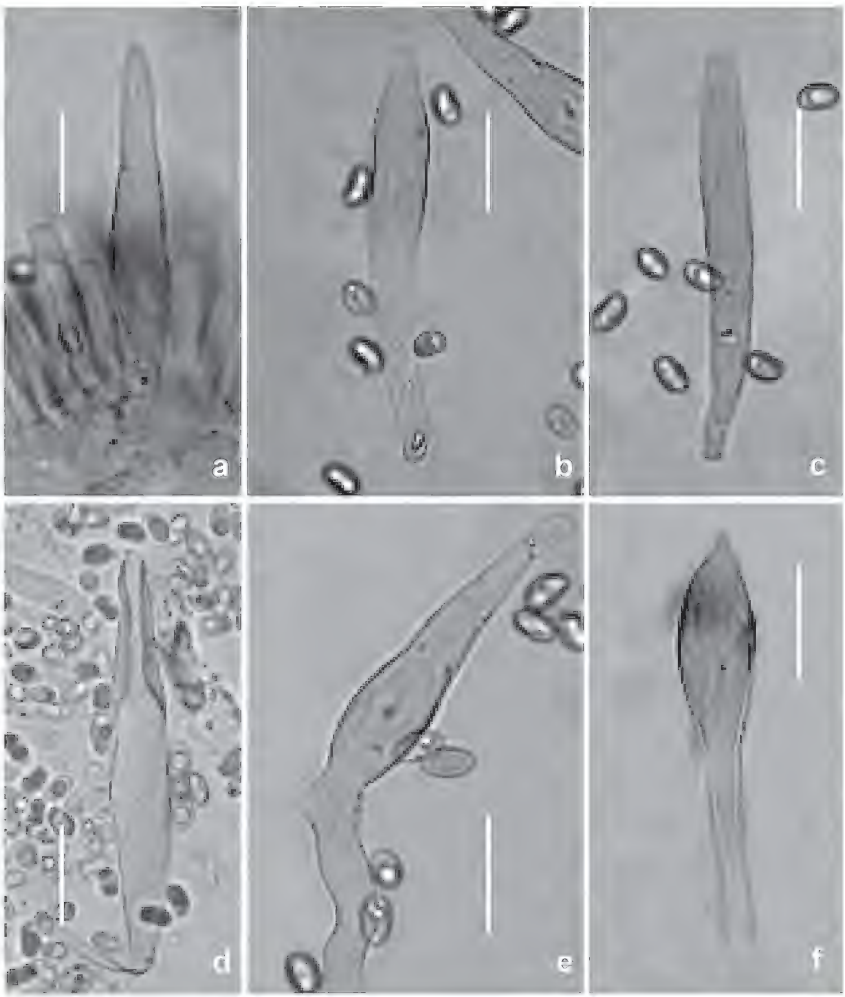

Fig. 4. *Phylloporus fagicola*. Pleurocystidia.
(a–c: Garay 215; d–f: del Moral 82, holotype). Bars = 20 μm.

subfusoid, thin-walled, other thick-walled up to 2.5 μm, or with incrustations, yellow in KOH, the measurements include mucronate subcylindrical or moderately ventricose sterile elements, 26–45 × 5–8 μm present at lamellae edges. Pileipellis a cutis of loosely intermixed hyphae when young, becoming as an interrupted layer of frequent mounds of chains of elements disposed in anticlinal orientation, loosely intermixed, with terminal cells clavate ventricose, subfusoid, subcylindrical, 12–55 × 3.5–7 μm, intercalary elements 12–38 ×

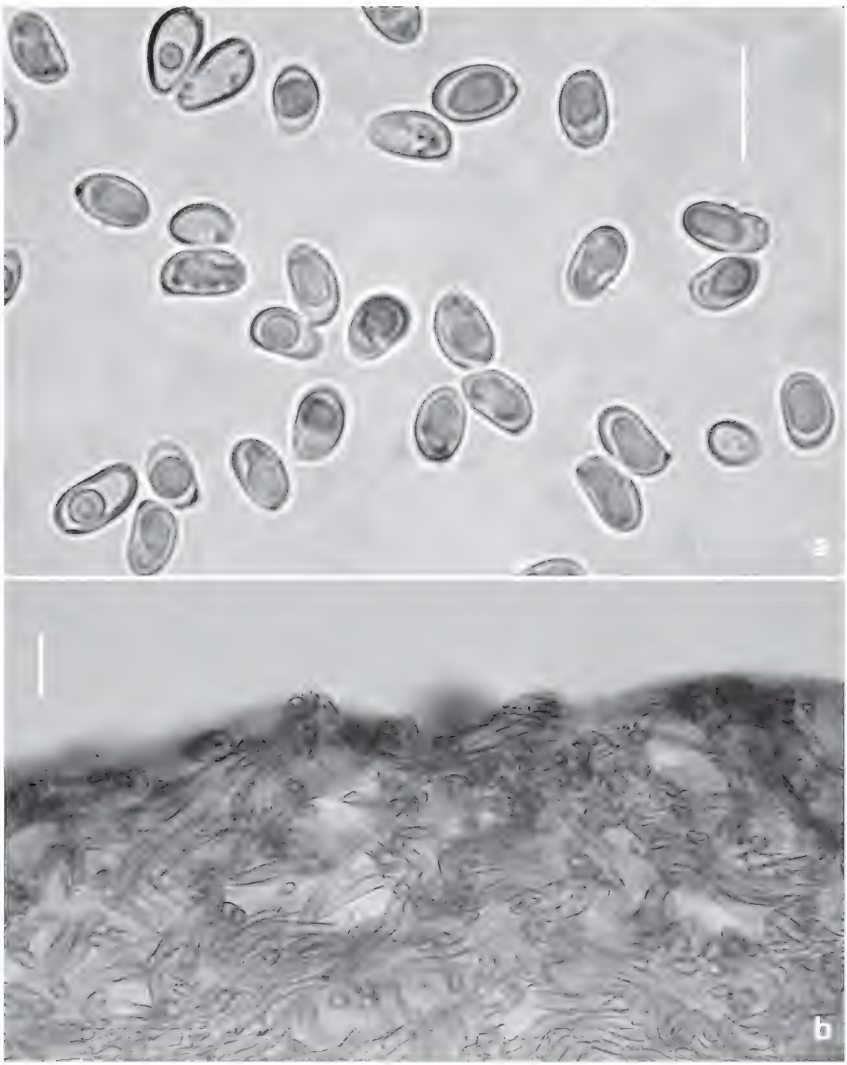

Fig. 5. *Phylloporus fagicola*. a. Basidiospores (Garay 215). b. Pileipellis (Montoya 4721). Bars: a = 10 μm, b = 20 μm.

3.5–6 μm, the mounds are variable in height and in some basidiomes arranged in a dense disposition, the hyphae appear more loosely arranged towards the context, all the elements yellow-amber in KOH, thin-walled. Context hyphae yellowish in KOH, hyphae loosely arranged 7–12 μm broad. Hymenophoral trama hyphae 3–12 μm broad, thin-walled, some with thick wall up to 2.5 μm

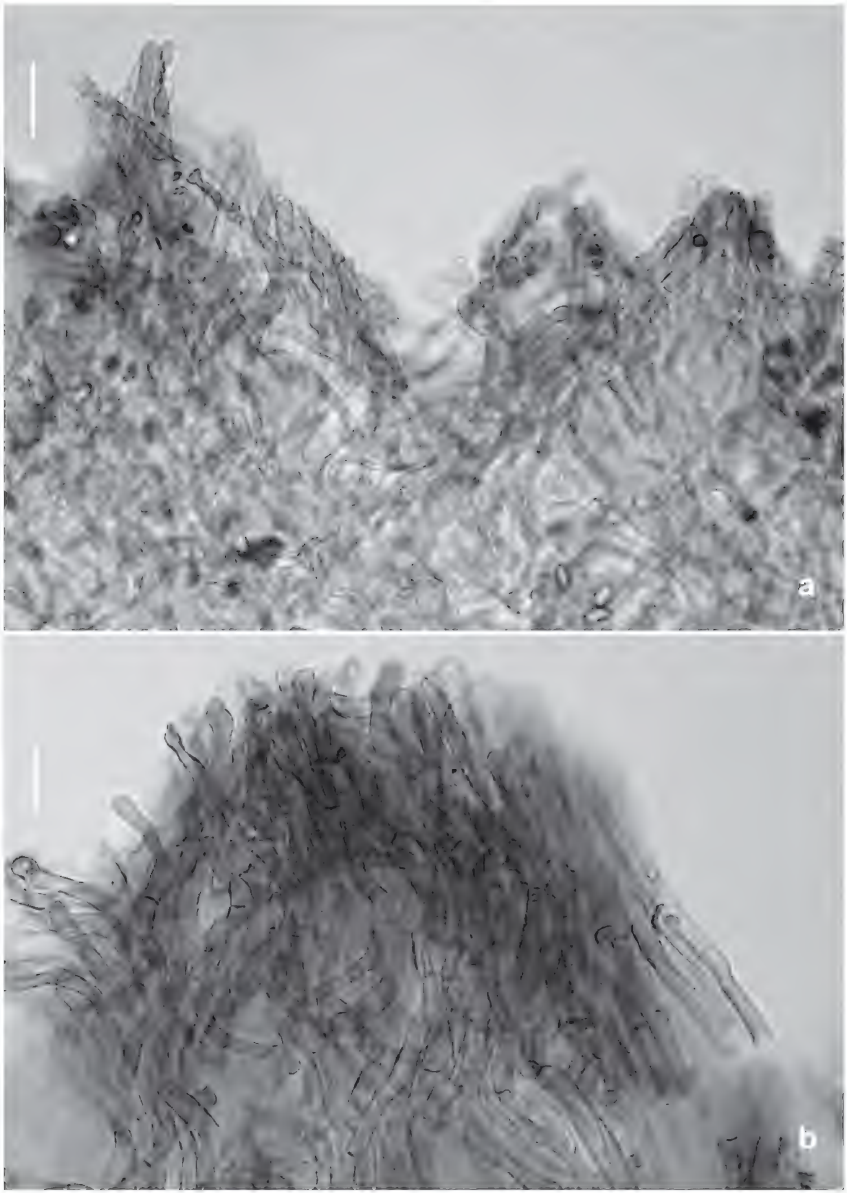

Fig. 6. *Phylloporus fagicola*, pileipellis (a: del Moral 82, holotype; b: del Moral 66). Bars = 20 μm.

or with incrustations, other with dense, refractive contents. The tissues exude an intense yellow sap when mounted in KOH.

Habitat — Gregarious in naked soil with dead leaves or with mosses in a *Fagus grandifolia* var. *mexicana* forest at 1840 m.

Additional specimens examined (all conserved at XAL): **MEXICO. Veracruz:** Mpio. Acatlán, Acatlán Volcano, August 14, 2007, del Moral 66; September 18, 2007, del Moral 117; September 10, 2008, del Moral 292; September 29, 2009, Garay 215. Mpio. Acajete, Mesa de la Yerba, August 8, 2006, Montoya 4606; October 22, 2008, Montoya 4721.

Discussion

Phylloporus fagicola is distinguished by the following features: a short, medium-sized basidiome, brightly colored (red-orange with yellow tones with egg-yellow to orange-yellow lamellae) but with context and surfaces bruising blue, crowded lamellae, thick-walled cystidia, and pileipellis at first a cutis and developing frequent mounds of anticlinal chains of elements when mature.

Among other *Phylloporus* taxa with ellipsoid to oblong basidiospores, *P. guzmanii* is distinguished by a vinaceous-red to dark purple pileus and stipe, gills that are more distant and lighter yellow, slightly narrower basidiospores [(6.4–)7.2–8.8(–10.4) × (3.2–)4.0–4.8(–5.6) µm], thin-walled and somewhat shorter cystidia [pleurocystidia (41.6–)45.0–105.6(–116.8) × 5.6–11.0(–12.0) µm; cheilocystidia 72.0–80.4 × 6.4–11.2 µm], and a trichodermial pileipellis with larger terminal elements [(20–)22–78(–80) × 5.5–10 µm]. *Phylloporus guzmanii* was recorded from Central and SW Mexico, growing in mesophytic *Quercus* and *Pinus* forests (Montoya & Bandala 1991).

Phylloporus aurantiacus, which possesses oblong basidiospores that are shorter (5.6–7.7 × 3.5–5 µm, X_m = 6.8 × 3.5 µm) and more ovoid in shape, is also distinguished by unchanging flesh when exposed, lamellae lacking red stains, shorter (60–90 × 7–10 µm) thin-walled cystidia, a trichodermial pileipellis, and an association with *Quercus* (Halling et al. 1999).

Phylloporus coccineus Corner from Singapore, also with broadly ellipsoid basidiospores that are somewhat shorter [7.5–9(–10) × 6.5–7.5(–8) µm] and more globose, differs from *P. fagicola* in subdistant gills, thin-walled larger cystidia [pleurocystidia 200 × 10–16 µm; cheilocystidia 70–120 × 10–18 µm], and a pileipellis comprising a compact palisade of terminal elements (25–40 × 5–10 µm) with reddish-orange sap and slightly thickened pink walls (Corner 1970).

During repeated surveys of both sites, the *P. fagicola* specimens fruited infrequently compared with other species recorded in the area. The new species appears to associate with *Fagus grandifolia* var. *mexicana* and has not yet been found in other montane cloud forest fragments of surrounding the area or in other regions explored by us.

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Type studies on two species of *Phylloporus* (*Boletaceae, Boletales*) described from southwestern China

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Abstract —Type specimens of two *Phylloporus* species originally described from southwestern China were studied. The concept of *P. luxiensis* is refined with additional recently collected materials, while *P. scabrosus* is revealed to be a member of the genus *Lentinus*.

Key words — morphology, revision, taxonomy

Introduction

Phylloporus Quél. is a diverse genus in the *Boletaceae*, the hymenophore of which is predominantly lamellate instead of poroid (Neves & Halling 2010). Thirteen species and one variety have been reported from China (Teng 1963; Zang & Zeng 1978; Li et al. 1992; Bi et al. 1993, 1994, 1997; Dai & Li 1994; Zang et al. 1996). However, some remain poorly known, especially *P. luxiensis* and *P. scabrosus*, both originally described from the southwestern region of the country (Zang & Zeng 1978). Only scanty information on microstructures was provided when the two species were first described with excellent vividly colored plates of basidiomata (Zang & Zeng 1978); although both taxa were later cited (Zang et al. 1996, Li & Song 2003), no additional morphological information was given. Because many *Phylloporus* collections have recently been made in the region, it is necessary to re-examine the types of the two species to characterize them in more detail. The species are described below based on critical examination of the holotypes and additional collections.

Materials & methods

The *P. luxiensis* and *P. scabrosus* holotypes and other *P. luxiensis* collections are deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese

Academy of Sciences (HKAS). For comparison, the holotype of *P. sulcatus* (Pat.) E.-J. Gilbert deposited in the Farlow Herbarium, Harvard University (FH) was also examined. 5% KOH was used as a mounting medium for microscopic studies. The notations "basidiospores (n/m/p)" indicate that the measurements were made on *n* basidiospores from *m* basidiomata of *p* collections. Dimensions of basidiospores are given using the notation (a–)b–c(–d); where the range b–c represents a minimum of 90% of the measured values, and extreme values (a and d) are given in parentheses. Q refers to the length/breadth ratio of basidiospores; Q_m refers to the average Q of basidiospores ± sample standard deviation. All line drawings of microstructures were made from rehydrated material. For the convenience of the readers, the original Latin protologue of the two taxa are provided.

Taxonomy

Phylloporus luxiensis M. Zang, Acta Microbiol. Sin. 18: 283, Pl. II: 1–4, 1978.

Figs. 1–3

Latin protologue — "*Pileus centralis, 5–6.5 cm latus, convexus demum plano-convexus vel concavus, siccus, griseo-umbrinus, subtestaceus vel griseo-fuscus. Stipes 1–1.5 × 5–6.5 cm, cylindricus vel basim versus attenuatus, concolor, spongioso-cavus, myceliis flavido-albis. Lamellae distantes, irregulares, decurrentes, flavidae. Basidiosporae ellipsoideae, subfuscoideae, 4–5 × 8.9–12.5 µm, laeves, hyalino-flavidae, 1-pleures guttulatae. Basidia 4-sporigera. Pleurocystidia numerosa, 12–16 × 25–35 µm, fusiformia, cylindracea vel late ellipticae, asperata, verruculosa. Cheilocystidia similia. Odor* [as "Oder"] *gratus et sapor subnullus.*" (Zang & Zeng 1978).

Type study — The holotype of *P. luxiensis* consists of a basidioma and a spore print wrapped in a paper possessing the following characters: Pileus 3.3 cm in diameter, convex; surface dry, yellowish brown, tomentose, margin inrolled. Hymenophore lamellate, decurrent. Lamellae distant, yellow, about 0.3 cm in height. Lamellulae common, concolorous with lamellae. Stipe central, 4.5 × 0.5 cm, cylindric, base slightly enlarged 1.2 cm; surface dry, with very small, reddish brown to yellowish brown, grayish brown squamules; the upper ribbed by the decurrent lines of the lamellae. Basal mycelium yellow. Annulus absent. Spore print pale yellowish brown.

Basidiospores [40/1/1] 10–12 × (4–)4.5–5 µm, Q = 2.00–2.44(–2.56), $Q_m = 2.26 \pm 0.15$, yellowish to yellowish brown in KOH, subfusiform to ellipsoid, slightly thick-walled (up to 0.7 µm), smooth under the light microscope, but with bacillate ornamentation under SEM (Figs.1 c–d, 2c). Pleurocystidia 51–67 × 12–17 µm, ventricose or subclavate, thin- to slightly thick-walled (up to 1 µm), colorless to yellowish in KOH (Fig. 2e). Hymenophoral trama hyphae 4–15 µm in diameter, thin-walled, colorless to yellowish in KOH. Basidia, Cheilocystidia and Pileipellis not recovered. Clamp connections not seen.

Study of additional collections — Basidiomata medium-sized (Fig. 1a–b, 2a). Pileus (2–)4–8 cm in diameter, convex, then applanate, center

FIG. 1. *Phylloporus luxiensis* (a from HKAS 57048; b from HKAS 52242; c–d from HKAS 57036). a–b. Basidiomata. c–d. SEM from dried specimen detailing bacillate ornamentation of basidiospores. Bars: a–b = 2 cm; c–d = 5 μm.

slightly depressed when old, but not infundibuliform; surface dry, brown, densely tomentose, covered with brown, cinnamon-brown to grayish brown squamules, margin inrolled; context white, unchanging in color when injured. HYMENOPHORE lamellate, decurrent. LAMELLAE moderately distant, up to 0.6 cm in height, occasionally anastomosing, yellow, dull yellow to ochre yellow, unchanging in color when injured. LAMELLULAE attenuate, concolorous with lamellae. STIPE central, 2–6 × 0.3–1 cm, cylindric, attenuate downwards, sometimes slightly enlarged at base (up to 1.4 cm), solid, firm; surface dry, upper half ribbed by the decurrent lines of the lamellae, with minute, reddish brown or purplish red squamules, lower half with yellowish brown, brown or grayish brown squamules; context white, unchanging in color when injured. BASAL MYCELIUM yellow. ANNULUS absent. ODOR indistinct.

BASIDIOSPORES [300/15/4] (8–)9.5–12.5(–14) × (4–)4.5–5(–5.5) μm, Q = (1.60–)1.90–2.70(–3.11), Q_m = 2.21 ± 0.25, yellowish to yellowish brown in KOH, smooth, subfusiform to ellipsoid, slightly thick-walled (up to 0.7 μm) (FIG. 2c). BASIDIA 33–44 × 9–10 μm, clavate, think-walled, 4-spored, hyaline to yellowish in KOH; sterigmata 4–5 μm long (FIG. 2d). HYMENOPHORAL TRAMA composed of thin-walled hyphae 3–14 μm in width, colorless to yellowish in

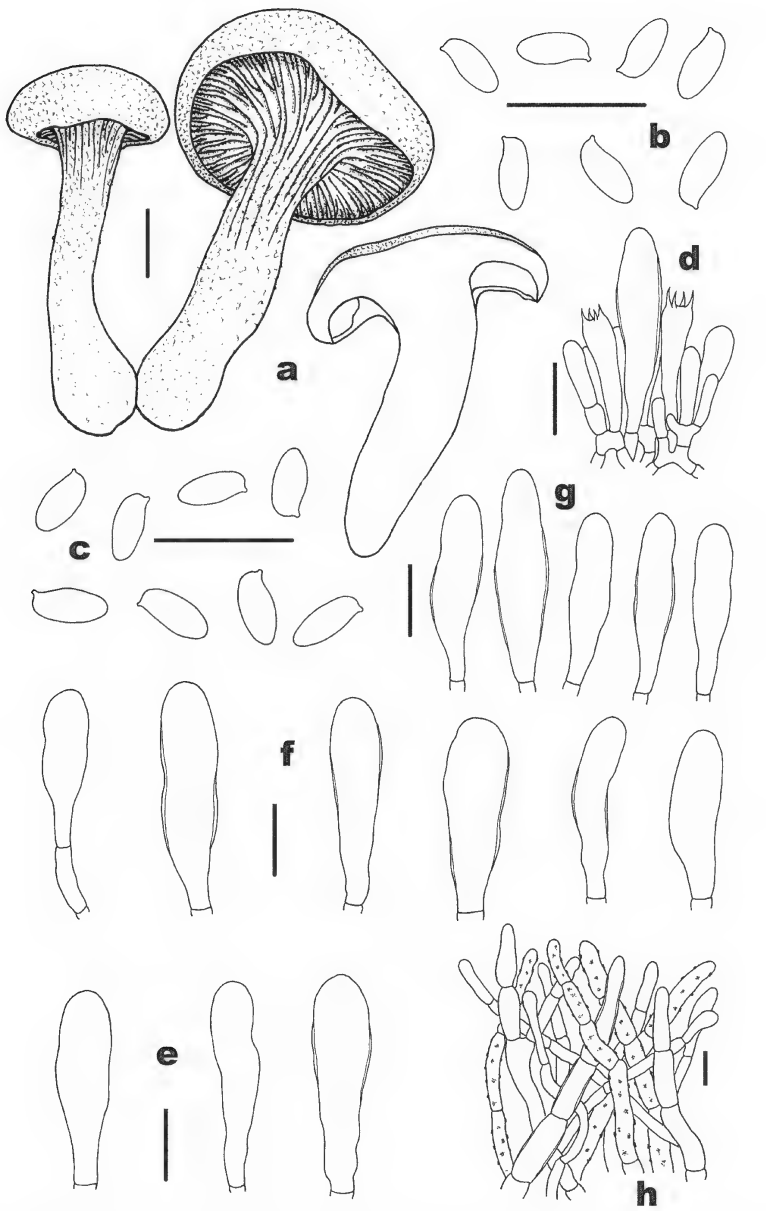

Fig. 2. Macroscopic and microscopic features of *Phylloporus luxiensis* (a from HKAS 57048; b, e from HKAS 40150, holotype; c–d, f–h from HKAS 57036). a. Basidiomata. b–c. Basidiospores. d. Basidia and pleurocystidium. e. Pleurocystidia. f. Cheilocystidia. g. Pleurocystidia. h. Pileipellis. Bars: a = 2 cm; b–h = 20 μm.

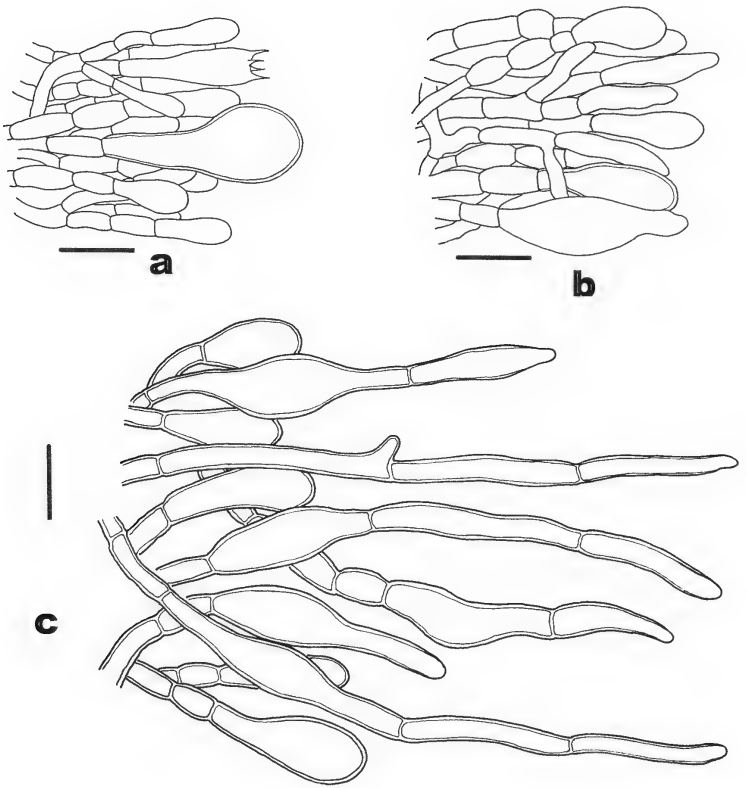

Fig. 3. Stipitipellis of *Phylloporus luxiensis* (HKAS 57036). a. From the apex of the stipe. b. From the middle part of the stipe. c. From the basal part of the stipe. Bars= 20 μm.

KOH. Cheilocystidia 36–65 × 10–19 μm, abundant, ventricose, subfusiform or subclavate, thin- to slightly thick-walled (up to 1 μm), colorless to yellowish in KOH, no encrustations (Fig. 2f). Pleurocystidia 42–105 × 10–19 μm, abundant, ventricose, subfusiform or subclavate, thin- to slightly thick-walled (up to 1 μm), colorless to yellowish in KOH, no encrustations (Fig. 2d,g). Pileipellis a slightly interwoven trichoderm composed of yellowish, 5–13 μm wide, thin- to slightly thick-walled (up to 1 μm), occasionally branching hyphae usually with yellowish granular encrustations, and clavate or subcylindrical terminal cells (20–62 × 5–12 μm) with attenuated or obtuse apex (Fig. 2h). Pileal trama composed of 4–12 μm wide, thin-walled, colorless to yellowish, interwoven hyphae. Stipitipellis a trichoderm-like structure; at the apex of the stipe composed of thin- to slightly thick-walled (up to 1 μm) hyphae with

narrowly or broadly clavate terminal cells (30–46 × 8–18 μm), and occasionally with clavate, 4-spored basidia (33–43 × 9–11 μm) (Fig. 3a); at the middle part of the stipe composed of thin- to slightly thick-walled (up to 1 μm) hyphae with clavate or subfusiform terminal cells (28–50 × 8–18 μm) (Fig. 3b); at the basal part of the stipe composed of slightly thick-walled (up to 1.5 μm) hyphae with broadly clavate or subfusiform terminal cells (30–71 × 14–20 μm), and many 3–20 μm wide, slightly thick-walled (up to 1.5 μm), emergent hyphae with clavate or subcylindrical terminal cells (33–50 × 5–9 μm) (Fig. 3c). Stipe trama composed of 4–10 μm wide, cylindrical, thin- to slightly thick-walled (up to 0.5 μm), colorless to yellowish in KOH, parallel hyphae. Clamp connections absent in all tissues.

Known distribution — Southwestern China.

Specimens examined — ***Phylloporus luxiensis***: **CHINA. Yunnan Province**: Mang City (previously called "Luxi County"), Chengguan Town, Xiadong, in the forest dominated by *Pinus* and *Quercus*, 3 July 1977, X.J. Li 90–HK2919 (HKAS 40150, **holotype**); Chuxiong Yi Autonomous Prefecture, bought from market, 26 August 2007, Z.L. Yang 4925 (HKAS 52242); Nanhua County, bought from market, 2 August 2009, L.P Tang 1079 (HKAS 57036); bought from market, 2 August 2009, L.P Tang 1080 (HKAS 57037); bought from market, 3 August 2009, L.P Tang 1091 (HKAS 57048).

Phylloporus sulcatus: **VIETNAM. Tonkin**: Vallée du Da-Pounian, 1907, Eberhardt LBA 210 (FH 3725, **holotype** of *Phylloporus sulcatus*), det. N.T. Patouillard.

Comments — In the protologue, the outer surface of the cell wall of cystidia was described as "verrucous". However, our observations differ from the original description.

Phylloporus luxiensis, originally described from Luxi County (now called "Mang City") in southwestern Yunnan, is well characterized by its brown, cinnamon-brown to grayish brown pileal surface, the reddish brown to yellowish brown, brown, or grayish brown stipe surface, the absence of staining of lamellae and context, and the non-inflated pileipellis hyphae.

Four collections cited above (HKAS 52242, 57036, 57037, 57048) matched well with the re-examined holotype and original illustrations. Our nuc-LSU and ITS sequence analyses (unpublished data) cluster all four collections together with a high statistic support. Unfortunately, no DNA sequences were successfully generated from the holotype.

Phylloporus sulcatus, a relatively unknown taxon described originally from Vietnam, shares some common characters with *P. luxiensis* (Patouillard 1909, Perreau & Joly 1964, Corner 1970). One of us (ZLY) studied the *P. sulcatus* holotype (FH–sheet 3725; Harvard University, Farlow Herbarium), which consists of the single collection, Eberhardt LBA 210. In the protologue of *P. sulcatus*, Patouillard (1909) cited a single locality, which corresponds exactly with the locality of Eberhardt LBA 210, which Singer (1945, 1978) therefore designated as the (holo)type. Eberhardt LBA 210 is comprises a single

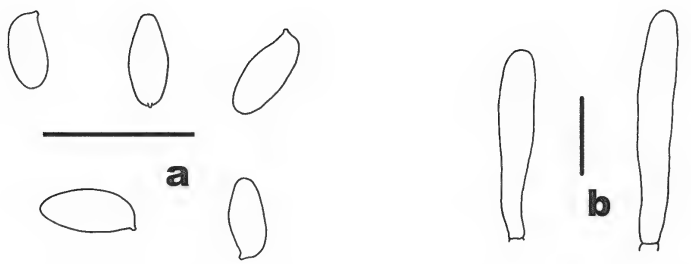

Fig. 4. Microscopic features of *Phylloporus sulcatus* (FH 3725, holotype). a. Basidiospores. b. Pleurocystidia. Bars = 20 μm.

basidioma. The dried cap is about 1.3 cm in diameter, convex, dark brown; the lamellae are decurrent, dark brown to blackish; the stipe is 2 × 0.2–0.3 cm, subcylindrical, dark, and its base is slightly enlarged; the basidiospores are [20/1/1] 10.5–12.5(–13) × (4.5–)5–5.5(–6) μm, Q = 2.0–2.36(–2.78), Q_m = 2.18 ± 0.19, yellowish to brown yellowish in KOH, smooth, ellipsoid (Fig. 4a); the basidia are 30–40 × 9–12 μm, clavate, 4-spored; the pleurocystidia are 55–70 × 9–12 μm, fairly abundant, subfusiform or narrowly clavate, with yellowish to brown yellowish vacuolar pigment, thin-walled, without encrustations (Fig. 4b). Based on our type study, *P. sulcatus* appears to differ from *P. luxiensis* in its wider basidiospores and narrower pleurocystidia. Furthermore, the stipe surface of *P. sulcatus* has no reddish tinge (Patouillard 1909, Perreau & Joly 1964, Corner 1970).

Phylloporus scabrosus M. Zang, Acta Microbiol. Sin. 18: 284, Pl. II: 5–8, 1978. Fig. 5

Latin protologue — "*Pileus centralis, 5–8 cm diam. convexus demum plano-convexus, siccus, rufobrunneus, chryseus vel subtestaceus. Stipes 1–1.5 × 8–10 cm, subcylindricus vel subclavatus, aequalis, sordide concolor, flexus, apice striatus, sursum scabroso-furfuraceus, basim versus squamuloso-furfuraceus. Lamellae distantes, decurrentes, flavido-albidae vel flavidae, myceliis flavido-testaceis. Basidiosporae subfuscoideae laeves, 5–5.5 × 11–12.5 μm, hyalino-flavidae. Basidia 4-sporigera. Pleurocystidia 10–15 × 25–35 μm, lageniformia vel fusiformia. Cheilocystidia non vidi. spongioso-carnosus. Odor* [as "Oder"] *gratus et sapor saccharatus.*" (Zang & Zeng 1978).

Type study — The *P. scabrosus* holotype comprises five basidiomata possessing the following characters: Pileus 3–4 cm in diameter, convex to applanate; surface dry, yellowish brown to chocolate brown, glabrous. Hymenophore lamellate, decurrent. Lamellae yellowish brown to dark brown, subdistant. Stipe central, 7.5–12 × 0.4–0.65 cm, cylindric, base slightly enlarged 0.9–1.2 cm, solid, firm; surface dry, concolorous with the pileus, velutinate, and also covered with disrupted squamules. Annulus absent. Texture very hard.

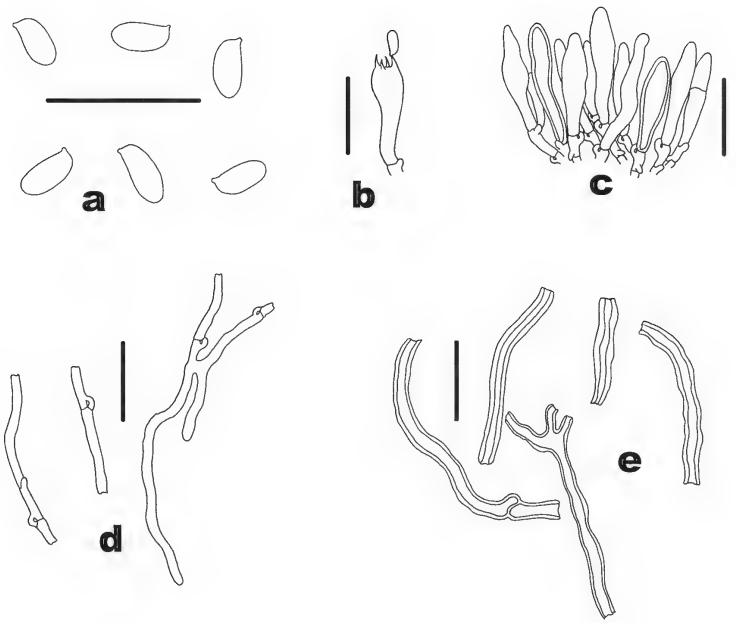

Fig. 5. Microscopic features of Phylloporus *scabrosus* (HKAS 28981, holotype). a. Basidiospores. b. Basidium. c. Cystidia. d. Generative hyphae. e. Skeletal hyphae. Bars = 20 μm.

Basidiospores [20/1/1] 6–8.5 × 3–4 μm, Q = 1.71–2.33, Q_m = 1.97 ± 0.20, yellowish brown in KOH, cylindric, smooth, thin-walled (Fig. 5a). Basidia 25–27 × 7–8 μm, clavate, yellowish brown in KOH, 4-spored (Fig. 5b). Cystidia 25–40 × 5–9 μm, finely abundant, subfusiform, cylindric or subclavate, thin- to slightly thick-walled (up to 1 μm), yellowish brown in KOH (Fig. 5c). Generative hyphae 3–5 μm in diameter, not inflated, yellowish to yellowish brown in KOH, frequently branching (Fig. 5d). Skeletal hyphae 5–13 μm in diameter, cylindric, yellowish brown in KOH, with a thickened wall (usually 2–3 μm thick, sometimes up to 5 μm thick), frequently branching (Fig. 5e). Clamp connections common in every part of the basidioma. Pileipellis and stipitipellis not recovered.

Specimen examined — **China. Yunnan Province**: Xishuangbanna Dai Autonomous Prefecture, Mengla County, Shangyong, Xinanshan, on fallen trunk, alt. 600 m, 24 September 1974, M. Zang 1640–HK1640 (HKAS 28981, **holotype**).

Comments — The five basidiomata of the holotype were probably not fully mature before being dried; only a few basidia with sterigmata and basidiospores were observed. The original protologue describes the basidiospores as measuring

11–12.5 × 5–5.5 µm (Zang & Zeng 1978), but our measurements differed. The holotype's basidiospores, basidia, and cystidia are filled with a yellowish brown necropigment, as observed in many other higher fungi (Locquin 1953; Yang 1997; Albee-Scott 2007; Petersen 2008a,b; Alvarado et al. 2010; Cripps et al. 2010).

Judging by the illustrations, texture, observed microscopic features, and lignicolous habit, this fungus apparently represents the genus *Lentinus* (see Corner 1981, Pegler 1983, Karunarathna et al. 2011).

Acknowledgments

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Arthrobotrys latispora, a new nematode-trapping fungus from southwest China

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Abstract — Using morphology and molecular phylogenetic analyses, we report a new nematophagous hyphomycete species, *Arthrobotrys latispora,* which produces erect, branched or unbranched conidiophores with conspicuous nodes at the tip; the conidia are nonseptate (41%) or uniseptate (59%) and broadly ovoid to oval. Three-dimensional adhesive networks formed in the presence of nematodes.

Key words — teleomorph–anamorph connection, *Orbilia*

Introduction

Nematode-trapping fungi have been the subject of decades-long research, including their ecology, distribution and systematics, due in part to their potential as biological control agents to manage plant and animal nematode diseases (Liu et al. 2002, Liu & Zhang 2003, Dong et al. 2004). These fungi are able to trap and consume nematodes with modified hyphae that include stalked and sessile adhesive knobs, adhesive nets, and constricting or non-constricting rings. Species forming adhesive nets are assigned to the anamorphic genus *Arthrobotrys* Corda, which currently comprises about 50 species (Scholler et al. 1999).

Pfister (1994) was the first to connect an *Arthrobotrys* species to the ascomycetous genus *Orbilia* on the basis of cultural studies. Up to now, six species of *Arthrobotrys* have been linked to related *Orbilia* teleomorphs. *Orbilia auricolor* (A. Bloxam) Sacc., seemingly a species complex, is known to have four anamorphs: *A. oligospora* Fresen., *A. cladodes* Drechsler (Pfister & Liftik 1995), *A. yunnanensis* M.H. Mo & K.Q. Zhang (Mo et al. 2005), and *A. psychrophila* (Drechsler) M. Scholler et al. (Rubner 1996). Of the remaining two species, *O.*

fimicola Jeng & J.C. Krug pairs with *A. superba* Corda (Pfister 1994) and an undescribed *Orbilia* sp. with *A. nonseptata* Z.F. Yu et al. (Li et al. 2009).

A survey of *Orbilia* species and their anamorphs revealed one *Arthrobotrys* species that had not yet been characterized. As neither morphological comparison nor phylogenetic analysis of the internal transcribed spacer (ITS) regions of rRNA genes could assign this isolate to a previously named taxon, it is reported here as a new species, *Arthrobotrys latispora*.

Materials & methods

Collection, isolation, and characterization of teleomorph fungi and their anamorphs

Fresh specimens of an *Orbilia* species were collected on decaying bark of *Castanopsis orthacantha* Franch. (*Fagaceae*), located in the northwest part of the YongPing County (25°7′18″–25°13′44″N 99°27′26″–99°36′07″E), Yunnan Province, China where the dominant plant species are *Castanopsis orthacantha*, *Lithocarpus variolosus* Chun, *Cyclobalanopsis glauca* Oerst., and *Cinnamomum glanduliferum* (Wall.) Meisn. Specimens are preserved in the herbarium of the Biology and Chemistry College of Dali University, China.

To cultivate the fungus, four apothecia were fixed to the lid of a Petri dish with hymenia positioned downward in order to collect discharged ascospores on the surface of CMA media (20 g corn meal, 18 g agar, 40 mg streptomycin, and 30 mg ampicillin in 1000 ml distilled water). These Petri dishes were placed at room temperature for 4–6 days until the ascospores germinated. Agar blocks with deposited ascospores were transferred to another CMA plate to avoid contamination and incubated at 25°C. After 10–15 days, the cultures were examined with an Olympus BX51 microscope with differential interference contrast. Trapping organs were induced by adding ~100 nematodes (*Panagrellus redivivus* Goodey) to a square slot (1 × 1 cm) created by removing the agar at the colony edge.

DNA extraction, PCR, and sequencing

Genomic DNA was extracted from the mycelia grown on cellophane membrane on PDA according to Jeewon et al. (2002). Primer pairs ITS5 and ITS4 (White et al. 1990) were used to amplify the complete ITS + 5.8S region. PCR amplification parameters were: 1 minute initial denaturation at 95°C, followed by 30 cycles of 1 minute denaturation at 94°C, 1 minute primer annealing at 50°C, 1.5 minutes extension at 72°C, and a final extension period of 10 minutes at 72°C. The purified PCR products were directly sequenced in forward and reverse directions using the same PCR primers (BGI Co., Ltd., China).

Phylogenetic analysis

Complete ITS1+5.8S+ITS2 rDNA sequences were aligned with 20 sequences (obtained from GenBank, NCBI [http://www.ncbi.nlm.nih.gov/]) by MUSCLE (MUltiple Sequence Comparison by Log-Expectation) at the EBI (European Bioinformatics Institute) web server [http://www.ebi.ac.uk/Tools/muscle/index.html]. ITS4 and ITS5 sequences were excluded from the analysis of all taxa. The DNA matrix of 21 taxa and 496 nucleotides was adjusted manually to improve the alignment. Phylogenetic analysis

was conducted with MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). Alignment gaps were treated as missing data; the sequence file set as interleaved; and GTR model was used for the substitution model. Markov chains were run for 1,000,000 generations, and trees were sampled every 10th generation resulting in 100,000 trees. About 0.003 average standard deviation of split frequency was achieved. The first 25,000 burn-in phase trees were discarded while the remaining 75,000 trees were used for calculating posterior probabilities in the consensus tree. The consensus tree was analyzed by the program FigTree v1.3.1. (Rambaut & Drummond 2010). The GenBank accession numbers of all the analyzed sequences were indicated after names of the species on the phylogenetic tree.

Results

Phylogenetic analysis

The Bayesian tree generated from the ITS1+5.8S+ITS2 rDNA sequences of *Arthrobotrys latispora* and its relative species (shown in Plate 1) indicates that *Arthrobotrys* and *Drechslerella* species group into two different clades, with *Dactylellina* species basal to both. The Bayesian posterior probabilities from the ITS genotyping resulting in these three major groups correspond to the different trapping device types and are consistent with the results of Scholler et

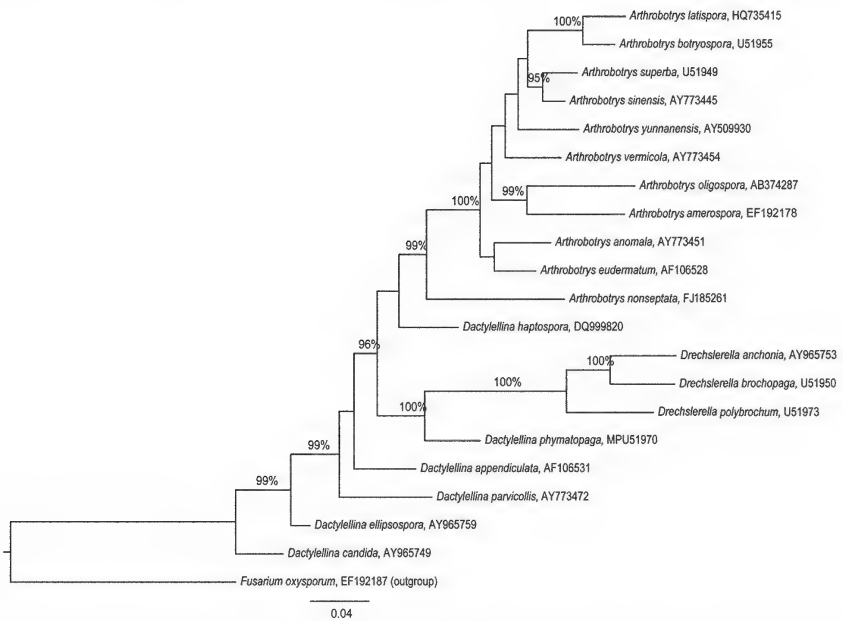

Plate 1. Bayesian tree derived from complete ITS1, ITS2 and 5.8S rDNA sequences of nematode-trapping fungi with *Fusarium oxysporum* set as outgroup. Bayesian posterior probabilities over 95% are shown on the branches, and scale bar at the bottom shows the expected changes per site.

Table 1. Morphology of *Arthrobotrys* species with non-septate conidia.

Species	Conidia			Conidiogen. denticle	Conidiophore (height µm)	Predacious organs
	Shape	Size (µm)	Septa (#)			
A. amerospora	obovoid	15–31 × 10–20	0	short	75–250	adhesive networks
A. anomala	cylindric to long ellipsoid	13–22 × 3–7	0 (–1)	short	20–80	adhesive branches & networks
A. arthrobotryoides sensu Drechsler	broadly cylindric-ellipsoid	17–30 × 10–16	(0–) 1	short	300–450	adhesive networks
A. botryospora	ellipsoid	12–20 × 11–15	0 (–1)	short	250–450	adhesive hyphae & networks
A. latispora	broadly ovoid-oval	14.8–21.5 × 10.1–16.3	0–1	short	60–250	adhesive networks
A. nonseptata	elongate ellipsoid	11–16.8 × 5–6.6	0	short	40–120	adhesive networks
A. yunnanensis	elongate, ellipsoid-cylindric, or clavate	17.5–32.5 × 2.75–7.5	0(–1)	long	60–200	adhesive networks

al. (1999). The trapping devices of *Arthrobotrys*, *Dactylellina*, and *Drechslerella* species form, respectively, adhesive networks, adhesive knobs, and constricting rings. *Arthrobotrys latispora* is situated within the adhesive networks clade and appears closely related to *A. botryospora* G.L. Barron. Morphological comparisons are shown in Table 1. Because our isolate differs from previously described *Arthobotrys* species, we propose it here as a new species.

Arthrobotrys latispora H.Y. Su & X.Y Yang, **sp. nov.** Plate 2

Coloniae in CMA effusae, ad 6 cm diam. post 8 dies 25°C. Mycelium sparsum, effusum, hyalinum, septatum, romosum, 2–4 µm laum. Conidiophora hyalina, simplicia, erecta, septata, non ramosa, plerumque 60–210 µm alta, basi 2–5 µm crassa, apice 1.8–4 µm crasso, efferenti 4–12 conidia sola de conidiogenis loci in perspicuis dendriculis in apicie aut prope apicem. Conidia hyalina, elongato ellipsoideo-cylindrica vel clavata, non- vel uniseptata, 14.8–21.5 × 10.1–16.3 (x = 18.99 × 13.5) µm. Reticula tenacia quae vermiculos nematodeos capiunt evolventibus. Chlamydosporae globosae vel ellipsoideae, catenulatae.

Holotype: Dried agar plate of a culture isolated from a collection of an undescribed *Orbilia* teleomorph [see below], (**holotype**, YMF 1.03168; ex-type culture, YMF 1.03168; **isotype** and ex-type culture, College of Agriculture and Biology, Dali University).

Etymology: '*latispora*' refers to the broadly ovoid conidia.

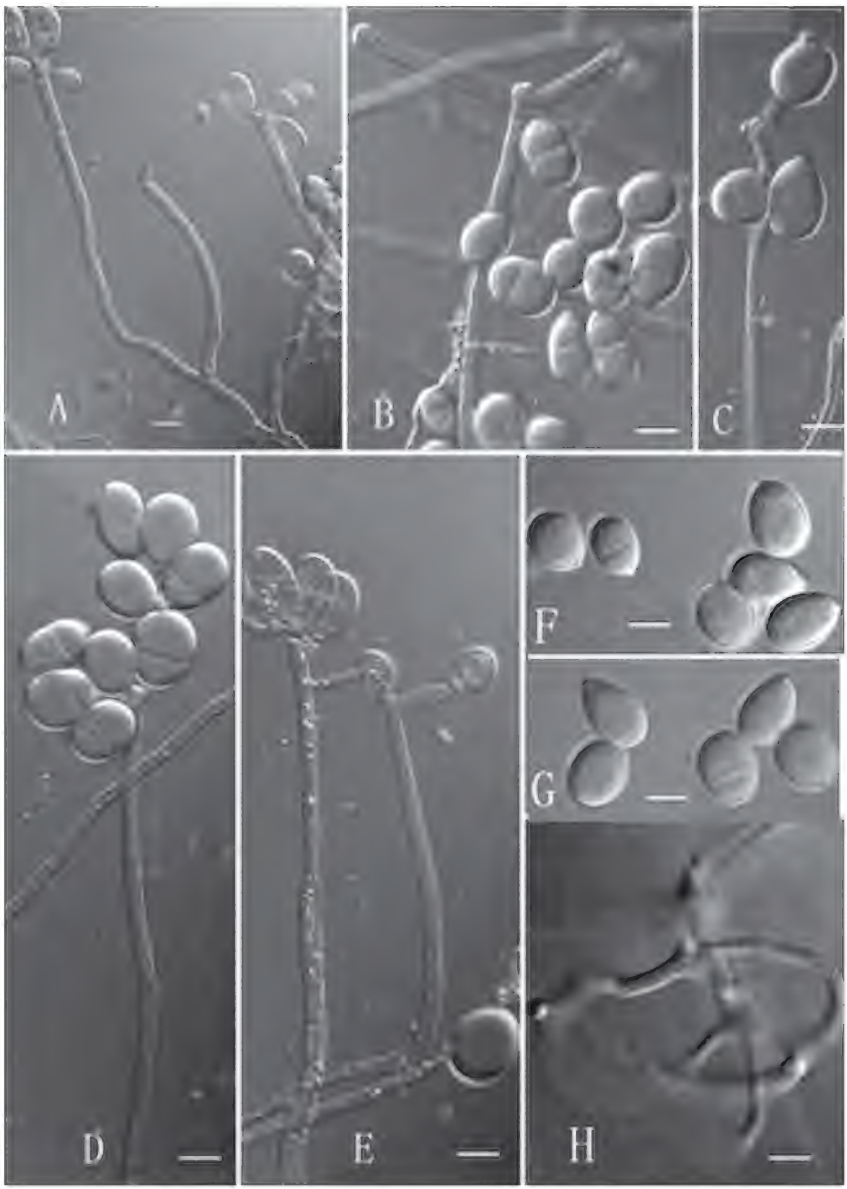

Plate 2. *Arthrobotrys latispora*:
A–E. Conidiophores with spore. F–G. Conidia. H. Adhesive nets. Bars: A–H =10 μm.

Assumed teleomorph: an unidentified *Orbilia* sp., YMFT 1.03168, collected on decaying bark of *Castanopsis orthacantha* (*Fagaceae*), PR CHINA, Yunnan Province, YongPing County, Jinguangsi Virgin Forest, alt. 2820 m, 20 september 2008, coll. X.J. Su, Z.L. Luo & H.Y. Su. (Duplicate material deposited at College of Agriculture and Biology, Dali University.)

Colonies grew rapidly on CMA medium, attaining 6 cm diam. in 8 days at 25°C. Mycelium spreading, vegetative hyphae hyaline, septate and branched, mostly 2–4 µm wide. Conidiophores colorless, erect, simple, septate frequently, 60–210 µm high, 2–5 µm wide at the base and 1.8–4 µm at the tip, producing 4–12 conidia singly from conidiogenous loci on conspicuous, partly superposed nodes at and near the apex. Conidia colorless, broadly ovoid-oval, broadly rounded at the tip, rounded truncate at the narrowed base, uniseptate or nonseptate at the center, 14.8–21.5 × 10.1–16.3 (x = 18.3 × 13.5) µm (living state). Approximately 41% of the conidia were nonseptate and 59% uniseptate. Chlamydospores spherical to ellipsoidal. Nematodes are trapped by three-dimensional adhesive networks.

Discussion

There are six known species of *Arthrobotrys* with nonseptate conidia, some of which form both uni- and nonseptate conidia. Conidia of *A. amerospora* (Schenck et al. 1977) and *A. nonseptata* (Li et al. 2009) are consistently nonseptate while those of *A. anomala* (Barron & Davidson 1972), *A. botryospora* (Barron 1979), and *A. yunnanensis* (Mo et al. 2005) are occasionally uniseptate. *A. arthrobotryoides* sensu Drechsler (1944), which consistently forms uniseptate conidia at 20°C, also forms many nonseptate ones at 28–32°C. Van Oorschot (1995) has noted that the identity of *A. arthrobotryoides* (Berl.) Lindau in its original sense cannot be determined from the protologue and cannot be resolved because of the absence of type material.

Arthrobotrys latispora is characterized by its broad, ovoid or slightly oval, partly nonseptate conidia, which are borne on nodes. Based on conidial shape, *A. latispora* most closely resembles *A. arthrobotryoides* sensu Drechsler and *A. amerospora*. The conidia of all three species are ovoid but differ in conidial size and septation. Differences between *A. latispora* and the other six known species that form non-septate conidia are summarized in Table 1.

Three additional collections also made on 20 September 2008 in the same region were associated with the same teleomorph but produced a very different anamorph referred to the genus *Anguillospora*. The question arises as to whether *A. latispora* is a contaminant, a conclusion supported by the fact that a similar, closely related teleomorph, *O. luteorubella* (Nyl.) P. Karst., also produced a similar *Anguillospora* anamorph (Pfister 1997).

Despite the nearly identical conidial morphology of *A. latispora* and *A. botryospora*, our molecular phylogenetic analysis pointed out a clear difference

between these two species: the ITS1+5.8S+ITS2 rDNA sequence similarity between *A. latispora* and a non-type isolate of *A. botryospora* is 93.7%. Thus *A. latispora* and *A. botryospora* can be considered two distinguishable species.

The phylogenetic analysis also showed that *Dactylellina* species were basal to both *Drechslerella* and *Arthrobotrys*. These results support the hypothesis of Li et al. (2005) that the trapping devices of *Drechslerella* species (with constricting rings) and *Arthrobotrys* species (with adhesive networks) developed independently from adhesive knobs (ancestral types of trapping devices specific to *Dactylellina*). Moreover, the analysis indicates that *Arthrobotrys* species may have evolved from *Dac. haptospora* relatives, whereas *Drechslerella* species evolved from *Dac. phymatopaga* relatives. More studies are needed to investigate the evolutionary process of these predacious fungi.

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Additional records of *Volvariella dunensis* (*Basidiomycota, Agaricales*): morphological and molecular characterization

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Abstract — Collections morphologically assignable to *Volvariella dunensis* from Sardinia and the Atlantic coast of Spain were revised and compared with the original collections. Molecular data supporting all examined collections as *V. dunensis* expand its known geographic distribution. A revised morphological characterization and a phylogenetic analysis of all *Volvariella* species sequenced to date are provided.

Key words — *Agaricomycetes*, ITS, phylogeny, *Volvariella volvacea*

Introduction

The genus *Volvariella* Speg., which is composed of saprotrophic or mycotrophic agarics, has been historically considered a member of the family *Pluteaceae* Kotl. & Pouzar (Singer 1986). The genus is characterized macroscopically by a pink spore-print, free lamellae, and a universal veil that forms a saccate volva at the base of the stipe and microscopically by the inverse hymenophoral trama (Singer 1986). Results from earlier molecular studies (Moncalvo et al. 2002, Matheny et al. 2006) led to questions about its monophyly and phylogenetic position in the *Pluteaceae*. More recent research focused on the *Pluteaceae* (Justo et al. 2011) showed that *Volvariella* is polyphyletic with two major clades. The large-spored, viscid species belonging to the *V. gloiocephala* complex were resolved as the sister group of *Pluteus* Fr. and accommodated under the new generic name *Volvopluteus* Vizzini et al. *Volvariella*, the remaining clade, is therefore restricted to the dry, small-spored species. *Volvariella* falls outside the pluteoid clade and based on data from ribosomal loci (nSSU, 5.8S, nLSU) shows affinities to some hygrophoroid

genera (*Cuphophyllus* (Donk) Bon, *Cantharocybe* H.E. Bigelow & A.H. Sm.), but its phylogenetic position within the *Agaricales* is still unclear.

Volvariella dunensis has been the subject of a detailed taxonomic study based on the re-examination of the original collections from the Mediterranean coast of Spain (Justo & Castro 2010). Very recently, a collection assignable to *V. dunensis* was reported from the Atlantic coast of Spain (Fernández-Sasia 2010 as "*Volvariella nigrovolvacea* var. *dunensis*"). Also, the identity of the Sardinian collection reported by Contu & La Rocca (1999) as *Volvariella nigrovolvacea* Kosina was recently questioned (Justo & Castro 2010), and it comes close to the morphological concept of *V. dunensis*.

The purpose of this study was to determine whether the collections reported by Fernández-Sasia (2010) and Contu & La Rocca (1999) represent *V. dunensis* using both morphological and molecular characters (ITS region).

Materials & methods

Morphology

Descriptive terminology follows Vellinga (1988) and Boekhout (1990). Microscopical preparations from dried material were mounted in Congo Red and 5% KOH. The following abbreviations are used in text: avl for average length, avw for average width, Q for quotient of length and width and avQ for average quotient. Extreme measurements are indicated within parentheses. Herbarium acronyms follow Thiers (2011) except that "SCM" is used for the "Societat Catalana de Micologia" herbarium and RFS refers to the personal herbarium of Roberto Fernández-Sasia.

Molecular analysis

Standard methods for DNA extraction, PCR amplification and DNA sequencing were applied (e.g. Justo et al. 2011). Primer pairs ITS1F and ITS4 (Gardes & Bruns 1993) were used for both PCR and sequencing. Complete information about the collections of *Volvariella* that were sequenced is given in Tab. 1. The remaining sequences used in the analysis were retrieved from GenBank and come from the studies of Menolli & Capelari (2008), Li et al. (2009) and Justo et al. (2011). GenBank accession numbers are given in Fig. 2. No suitable outgroup for *Volvariella* was found using BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Therefore, after running a preliminary analysis using *Coprinus comatus* (O.F. Müll.) Pers. as an outgroup for all *Volvariella* sequences and taking into consideration the results from the combined nLSU-5.8S-nSSU analyses of Justo et al. (2011), *V. bombycina* (Schaeff.) Singer and *V. volvacea* (Bull.) Singer were used as outgroup taxa in the final dataset. These two species appear to be the sister-group to all other species of *Volvariella* sequenced to date. Sequences were aligned with MAFFT (Katoh et al. 2002) using the Q-INS-i strategy. The alignment was examined and manually corrected in MacClade 4.05 (Maddison & Maddison 2002). It has been deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S11441). Pairwise similarities were calculated using Jalview (Waterhouse et al. 2009). A Maximum Likelihood analysis was run in RAxML servers (Stamatakis et al. 2008) with 100 rapid bootstrap replicates.

Table 1. *Volvariella* samples newly sequenced in this study.

Species	Country—location	Collector; sampling date [herb. number]	Habitat
V. dunensis (Vila et al. 1999, Justo & Castro 2010)	Spain—Barcelona, El Prat de Llobregat, El Pinar	F. Àngel; 08.II.1997 [SCM 3513]	Open dunes
V. dunensis (Fernández-Sasia 2010)	Spain—Bizkaia, Muskiz	R. Fernández-Sasia; 10.III.2007 [RFS 070310-03]	Dunes with *Tamarix gallica* & *Carpobrotus edulis*
V. dunensis (Contu & La Rocca 1999 as *V. nigrovolvacea*)	Italy—Sardinia, prov. Cagliari, Chia	M. Contu; 26.XII.1997 [TO-AV140]	Sand dune in *Juniperus phoenicea* debris; among *Pistacia lentiscus*, *J. phoenicea*, *J. oxycedrus*
V. hypopithys (Fr.) Shaffer	Italy—Stazzo Montesu, Olbia-Tempio	M. Contu; 26.XI.2002 [TO-AV137]	Under *Quercus suber*
V. pusilla (Pers.) Singer	Spain—Asturias, Endriga	R. Picón; 5.X.2005 [AJ51 (LOU)]	Among grasses
V. pusilla	Italy—Oschiri, Olbia-Tempio	M. Contu; 11.XI.2000 [TO-AV139]	Among grasses
V. strangulata (Romagn.) Vizzini & Contu	Italy—Emilia Romagna	L. Setti; — [TO-AV141]	Not recorded
V. terrea	France—Rixheim	E. Musumeci; 01.X.2005 [LUG 11010, holotypus]	Under *Carpinus betulus* near *Agaricus xanthodermus*
Volvariella sp.	Spain—Madrid, Parque del Oeste	L.A. Parra; 6.VIII.2005 [LOU 18294]	Among *Cedrus atlantica* remnants
Volvariella sp.	Slovenia—Tolminski, Tolmin	J. Kavcic; 12.VII.2007 [TO-AV143]	Under *Fagus sylvatica* and *Fraxinus* sp.

Taxonomy

ITS sequences from the collections TO-AV140 (Sardinia) and RFS 070310-03 (Atlantic coast of Spain) are 99.5% and 100% identical to the ITS of SCM 3513 (Mediterranean coast of Spain), respectively. All collections are therefore considered to represent *V. dunensis*.

Macromorphological differences between the Sardinian and Atlantic collections with respect to the original collections of *V. dunensis* are subtle, but they concern characters commonly used to delimitate species in *Volvariella* such as pileus diameter, stipe colour and volva morphology and colour. Variation in

FIGURE 1. *Volvariella dunensis*. Basidiomes (Sardinia, Italy, TO AV140). Bar = 50 mm.

microscopical characters is also subtle and mainly concerns size and shape of basidiospores and cystidia. Here we provide a revised description of *V. dunensis* based on all the collections known so far, highlighting only those characters that differ from the description provided in Justo & Castro (2010).

Volvariella dunensis (Vila, Àngel & Llimona) Justo & M.L. Castro, Mycotaxon 112: 262. 2010. FIG. 1

≡ *Volvariella nigrovolvacea* var. *dunensis* Vila, Àngel & Llimona, Revista Catalana de Micologia 22: 131. 1999.

PILEUS diameter 15–100 mm. STIPE surface white to grey (similar to pileus colour). VOLVA white to externally dark sepia to brownish-black, entire or lobate. BASIDIOSPORES (6–)6.5–9.0 × 4.5–6(–6.7) µm, avl × avw = 7.5–7.9 × 5.1–5.5 µm, Q = (1.15–)1.3–1.7(–1.8), avQ = 1.4–1.55, ellipsoid to oblong, rarely broadly ellipsoid. PLEUROCYSTIDIA (34–)40–95(–112) × 13.5–45(–50) µm, clavate, (narrowly) utriform, obovoid, more rarely broadly lageniform or fusiform; colourless; with thin, smooth walls, a few with a slightly thickened wall (up to 0.8 µm thick). CHEILOCYSTIDIA 20–80(–120) × 15–60 µm, clavate, utriform, fusiform or lanceolate, without apical appendages, colourless; with thin, smooth walls, a few with a slightly thickened wall (up to 0.8 µm thick). CAULOCYSTIDIA only observed in the original collections, absent in TO-AV140 and RFS 070310-03.

HABIT, HABITAT AND DISTRIBUTION — Gregarious, usually with most of the basidiomes growing deeply buried in the sand. In coastal dunes. Known from the Mediterranean basin (NE Spain, Sardinia) and the Atlantic coast of Spain.

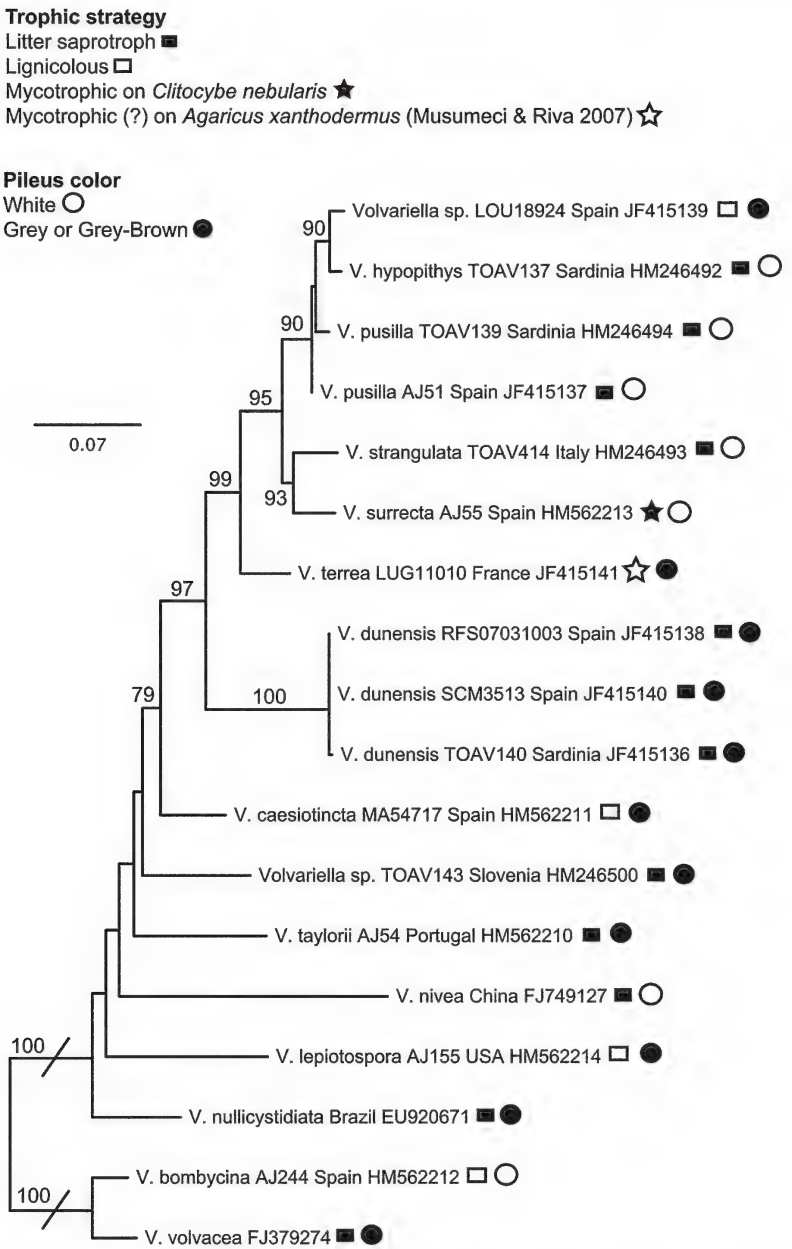

FIGURE 2. Best tree from the Maximum Likelihood analysis of ITS sequences of *Volvariella*. Bootstrap values above 70% are indicated on the branches. The root length has been reduced to facilitate graphical representation. Scale bar value indicates nucleotide substitutions/site.

Phenology — *Volvariella dunensis* seems to have a relatively narrow and particular time of fructification during the winter, spanning from the last days of December (Sardinia) to early March (Atlantic coast of Spain).

Comments—The arenicolous habitat, winter fructification, and cystidial size and morphology are the key characters for separating *V. dunensis* from other species with grey-fibrillose pilei, especially *V. volvacea*. Courtecuisse (1984) reports that *V. volvacea* could be collected "au niveau des dunes" in the Atlantic coast of France, and Bon & Gehu (1973) signal this species as typical of the *Ammophiletea* communities. In the UK (Orton 1986), Netherlands (Boekhout 1990), and Spain (Justo & Castro 2010), *V. volvacea* has been consistently reported as growing inside greenhouses or outside on organic rich substrates (leaves, compost, sawdust). Given the external resemblance of both species it is very likely, in our opinion, that at least some coastal records of *V. volvacea* from France may, in fact, represent *V. dunensis*.

In the phylogenetic analysis, *V. dunensis* appears as sister to the clade containing *V. terrea* Musumeci & A. Riva and the *V. pusilla* group (Fig. 2). Apart from this relationship, the internal nodes of the tree receive generally low support and many species appear on relatively long branches. This is probably a consequence of the high level of divergence in the ITS sequences of *Volvariella*. For instance, the relatively low 62% pairwise similarity between *V. bombycina* and *V. nivea* T.H. Li & Xiang L. Chen could indicate either a high rate of molecular change in the genus or a relatively ancient speciation event.

The number of *Volvariella* taxa and collections sampled for molecular analyses is still insufficient to make broad generalizations about the morphological species-rank taxonomy. However, it seems that many characters traditionally used for grouping species in the genus such as pileus colour (grey-brown vs. white) or trophic strategy (litter saprotroph, lignicolous, mycotrophic) have undergone many transitions during the evolution of *Volvariella* species (see character states on Fig. 2). Additional research using both morphological and molecular data is badly needed to gain further insights in the taxonomy and evolution of the genus.

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Moelleriella pumatensis, a new entomogenous species from Vietnam

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Abstract — *Moelleriella pumatensis*, a fungal pathogen infecting scale insect nymphs (*Hemiptera*), is described and illustrated as a new species from Pu Mat National Park in Vietnam. This species is unique in producing a golden yellow spore mass surrounding the stroma. In surveys throughout the year in Vietnam, only the anamorphic state has been found in the natural forest. Morphological characters and phylogenetic analysis of translation elongation factor 1-α (*tef1*) reveals this species as an anamorph of *Moelleriella*.

Key words — morphology, phylogenetics, taxonomy

Introduction

The genus *Moelleriella* Bres. (*Ascomycota, Hypocreales, Clavicipitaceae*), a fungus pathogenic to scale insects and white flies, was recently segregated from the genus *Hypocrella* Sacc. together with *Samuelsia* P. Chaverri & K.T. Hodge (Chaverri et al. 2008). *Moelleriella* was described based on molecular data and morphology: its ascospores disarticulate inside the ascus, whereas *Hypocrella* and *Samuelsia* ascospores do not. The anamorphic state of *Moelleriella* is *Aschersonia*-like, i.e., similar to *Aschersonia* sensu stricto (teleomorph *Hypocrella* sensu lato; Chaverri et al. 2008). *Aschersonia* sensu lato species are differentiated mostly on the shape and color of the stromata that cover the hosts, pycnidium-like conidiomata, phialides, and presence or absence of paraphyses. These characters have been useful in distinguishing between subgenera of *Aschersonia* (Petch 1921, 1925, Mains 1959, Chaverri et al. 2008). All species of *Hypocrella*, *Moelleriella,* and *Samuelsia* are pathogenic to scale insects (*Coccidae*) or white flies (*Aleyrodidae*) that feed on living leaves and branches of monocotyledonous and dicotyledonous plants.

Exploration of entomopathogenic fungi in Vietnam was initiated under the program of biodiversity studies in Southeast Asia, a collaborative project between the Faculty of Agriculture, Forestry and Fisheries, Vinh University, Vietnam, and the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand. One study site, Pu Mat National Park, is located in the southwest of Nghe An Province, an area known to have a high diversity of plants and mammal species. During the surveys in October 2009, an interesting *Aschersonia*-like specimen that produced a yellow mass of conidia covering the stroma was collected from the underside of a leaf of a dicotyledonous plant. The objectives of this study were to 1) evaluate the taxonomic position of the isolates derived from the anamorph state, using the sequences of translation elongation factor 1-α (*tef1*), and 2) describe this specimen as a new species from Vietnam.

Materials & methods

Collection and Isolation

Surveys and collections were made during the rainy season in the Khe Moi trail in Pu Mat National Park (18°46′–19°12′N 104°24′–104°56′E). Material was examined and isolated into pure culture from the anamorphic state following Mongkolsamrit et al (2009). Free-hand longitudinal sections of tissues and conidia were mounted in cotton blue in lactophenol (Heritage et al. 1996) and measured using a light microscope. The color of fresh specimens and cultures were compared with the colors from standard code from Kornerup & Wanscher (1962). Specimens were identified using specialized literature (Petch 1921, Mains 1959, Chaverri et al. 2008). A voucher specimen and culture were deposited in BIOTEC Bangkok Herbarium (BBH) and BIOTEC Culture Collection, Thailand.

DNA extraction, amplification and sequencing

Small pieces of tissue (2–3 mm) of each sample were taken from potato-dextrose-agar (PDA) plates and placed in 50 ml of potato-dextrose-broth (PDB) in a 250 ml flask. Flasks were incubated in a shaker at 200 rpm for 15–20 d at 20°C in dark. The mycelial mass was harvested by filtration (Whatman No.1), washed with sterile distilled water, lyophilized for 1 d, and crushed in liquid nitrogen using a mortar and pestle. Total DNA of each sample was extracted using Cetyltrimethyl-ammonium bromide (CTAB) following the procedure described in Mackill & Bonman (1995). PCR amplification was done in 50 µl volume consisting of 1× PCR buffer, 200 µM of each of the four dNTPs, 2.5 mM $MgCl_2$, 1 U Taq DNA polymerase (Promega, Madison, Wisconsin) and 0.5 µM of each primer. The primers for *tef1* were 983f (Carbone & Kohn 1999) and 2218r (Rehner 2001). Amplifications were performed using a MJ Research DNA Engine ALD1244 thermal cycler following the procedure described in Sung et al. (2001). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's instructions. Purified PCR products were sent to Macrogen Inc. Korea for sequencing.

Table 1. List of fungi used in this study.

Species	Voucher/Isolate	GenBank
Balansia henningsiana	GAM 16112	AY489610
Epichloe elymi	C. Schardl 760	AY986951
Hypocrella disciformis	M.L.202i = ARSEF 7695	AY986939
H. disciformis	P.C.655 = CUP 067861	EU392643
H. disciformis	P.C.676 = CUP 067840	EU392645
H. viridans	I89-490 = IMI 346739	EU392649
H. viridans	P.C.632 = CUP 067849	EU392650
Moelleriella mollii	I93-901a = ARSEF 7660	EU392667
M. mollii	I93-901c = ARSEF 7667	EU392668
M. ochracea	IE1308 = P.C.726	EU392669
M. ochracea	P.C.535 = CUP 067777	AY986926
M. ochracea	P.C.626 = CUP 067778	EU392670
M. ochracea	P.C.648 = CUP 067779	EU392671
M. pumatensis	BBH 29281 = BCC 41004	HQ722026*
M. pumatensis	BBH 29281 = BCC 41006	HQ722027*
Samuelsia chalalensis	P.C.560 = CUP 067856	EU392691
S. geonomis	P.C.614 = CUP 067857	EU392692
S. rufobrunnea	P.C.613 = CUP 067858	AY986944

*sequences generated in this study.

Phylogenetic analysis

Sequences were proofed manually, assembled using BioEdit v. 6.0.7 (Hall 2004), and submitted to Genbank (Table 1). Sequences were aligned using ClustalW incorporated in BioEdit and alignments were refined manually by direct examination. Maximum parsimony analysis was performed using random addition sequence (10 replications) and gaps were treated as missing data. Bootstrap analysis was performed using maximum parsimony criterion in 1000 replication samples.

Results

Molecular analysis

Amplification and sequencing of *tef1* were successful for 16 strains comprising two unidentified *Aschersonia*-like specimens. Fourteen selected closely related taxa were obtained from GenBank for this study. Sequences of *Epichloe elymi* and *Balansia henningsiana* were used as outgroup taxa. Of the 899-character alignment in the *tef1* data set, 188 characters were parsimony informative. Maximum parsimony analyses of this data set yielded one parsimonious tree (tree length 436; CI = 0.651, RI = 0.795, RC = 0.518, HI = 0.349) as shown in Fig 1. The phylogenic tree suggests that the two anamorphic isolates with yellow conidial masses (BCC41004 and BCC41006) belong to the genus *Moelleriella* with a strong bootstrap support of 99%.

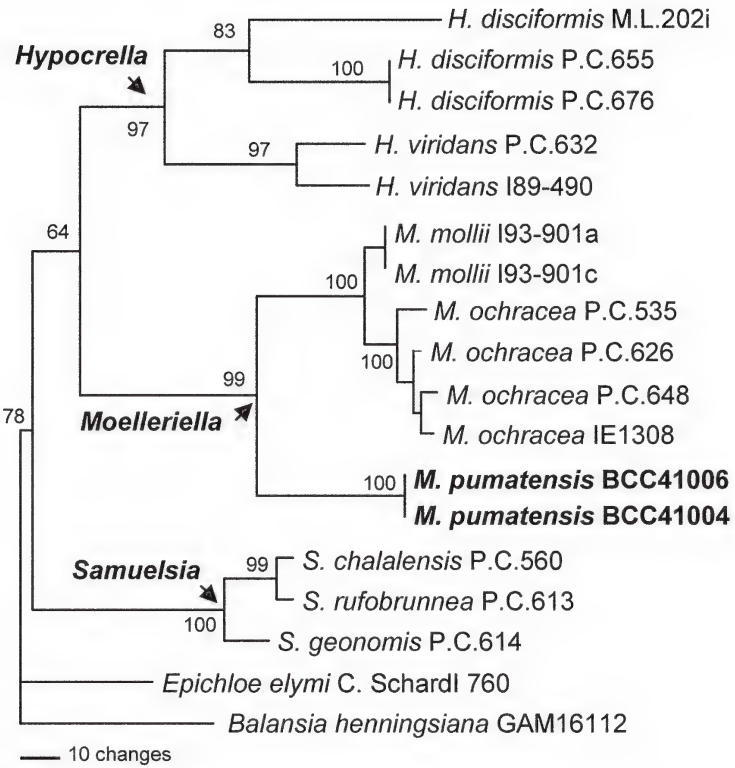

FIG. 1. Phylogenetic relationship of *Moelleriella pumatensis* and related species based on maximum parsimony analysis of the TEF1 gene. Numbers above each branch represent bootstrap support from 1000 replicates.

Taxonomy

Moelleriella pumatensis T.T. Nguyen & N.L. Tran, sp. nov. FIG. 2

MYCOBANK MB 561074

Stromata discoidea vel pulvinata, ad 2.5 mm diam, 1.5 mm alta, sursum rotundata, aureus, Conidiomata pycnidialia, immerse, disperses, 250–280 µm alta, 90–100 µm diam. Phialides cylindricae, ad 25 µm longae. Paraphyses pycnidiales praesentes, filiformes, flexuosae, ad 180 µm longae, 1.5 µm latae. Conidia fusoidea, utrinque acutata 12–15 × 2–2.5 µm.

TYPE — Vietnam: Nghe An Province, Khe Moi trail, Pu Mat National Park, on scale insect nymph (*Hemiptera*) on the underside of dicotyledonous leaf, 18 Oct. 2009, J.J. Luangsa-ard, S. Mongkolsamrit, T.T. Nguyen, R. Ridkaew & N.L. Tran (BBH 29281, **holotype**; ex-type culture BCC41006).

ETYMOLOGY — referring to Pu Mat National Park, the collection location.

ANAMORPH: *Aschersonia*-like

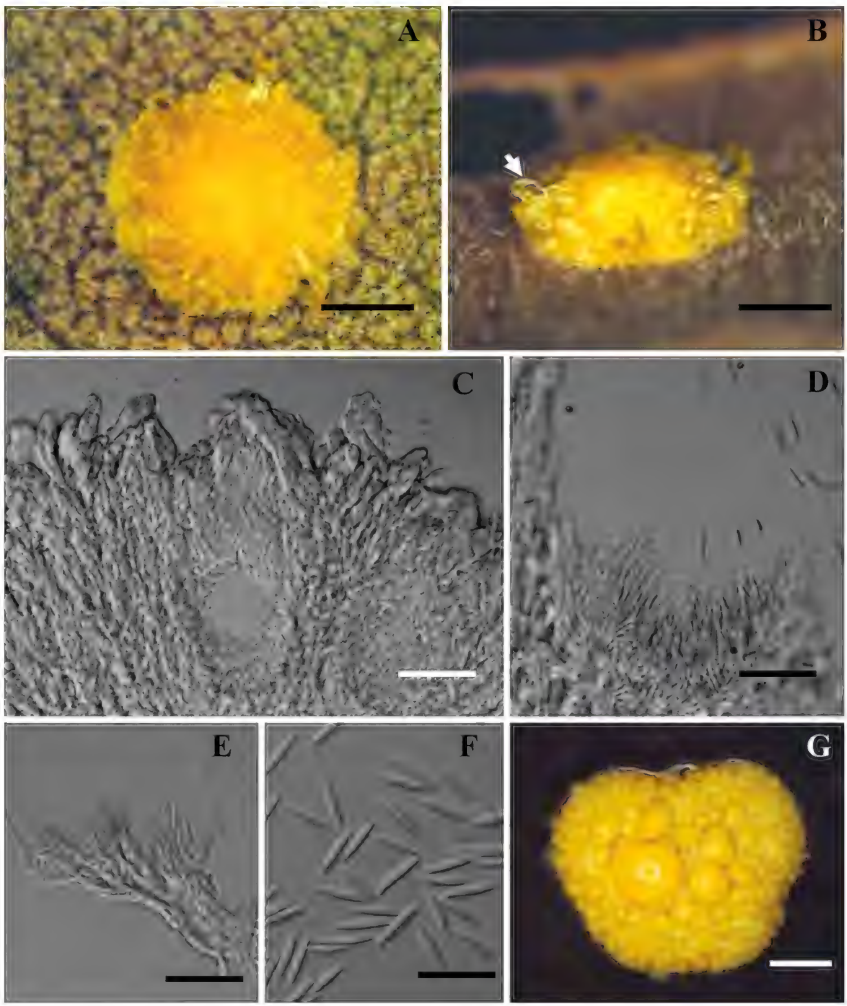

Fig. 2. *Moelleriella pumatensis* A, B, stroma on host showing conidia mass (white arrow); C, pycnidium and sporulating structure; D, E, conidiogenous cells and paraphyses; F, conidia; G, colony on PDA at 20°C after 4 wk (sporulation present). Scale bars: A, B, G = 1 mm; C = 100 µm; D, E, F = 20 µm.

Stromata discoid to pulvinate up to 2.5 mm diam and 1 mm high, upper surface round, golden yellow (Kornerup & Wanscher 1962: 5B7). Conidiomata pycnidial, embedded, scattered in stroma, ovoid, 250–280 µm high, 90–100 µm diam. Conidiogenous cells phialidic, cylindrical, up to 25 µm long. Pycnidial paraphyses present, linear, filiform, flexuous, up to 180 µm long, 1.5 µm wide. Conidia narrowly fusiform, with acute ends, 12–15 × 2–2.5 µm.

Cultural characteristics: Conidia germinating within 24 h on PDA. Colonies on PDA slow-growing, attaining 5 mm diam in 4 wk. Optimal temperature 20–25°C, with no growth at <5°C and >35°C. Stromatic colonies yellow, forming moderately compact stromata. Conidial masses golden yellow (Kornerup & Wanscher 1962: 5B7), appearing as abundant slimy masses from immersed pycnidia scattered over the surface.

Comments: A teleomorphic state of this species was not found in the field although several attempts to find it were made throughout the year. However, the teleomorph name is used for this new species because both phylogenetic analysis and morphology support the placement of this species in *Moelleriella.* In this case, as well as for *Moelleriella madidiensis* from Bolivia also with only an anamorphic state, the teleomorphic name is used (Chaverri et al. 2008). The anamorphic state of *M. pumatensis* is similar to *Aschersonia aleyrodis* (teleomorph: *M. libera*) reported from Brazil, Florida, and Venezuela by Petch (1921) based on pulvinate stroma, yellow mass of extruded spores, and fusiform conidia. The conidia and paraphyses of *M. pumatensis* are somewhat longer; the conidia are 12–15 × 2–2.5 µm and paraphyses up to 180 µm long. In contrast, *A. aleyrodis* has conidia 8–14 × 1.5–2 µm, paraphyses 50–110 µm long. In addition, *M. pumatensis* was compared morphologically with several species collected from the Neotropics (Chaverri et al. 2008), China (Qiu et al. 2009, Qiu & Guan 2010), and Thailand (Luangsa-ard et al. 2007, 2008, 2010, Mongkolsamrit et al. 2009). The Vietnamese specimens are mostly similar to *Aschersonia andropogonis* (teleomorph: *M. ochracea*) in having pulvinate stroma, yellow, yellowish orange to orange spore masses, fusiform conidia (8–14 × 1.5–2 µm), which is commonly found in the Neotropics. However, *M. pumatensis* differs from *A. andropogonis* in the color of the stroma. *Moelleriella pumatensis* has a golden yellow stroma while that of *A. andropogonis* is white to pale yellow. The DNA sequence analysis of translation elongation factor 1-α (*tef1*) also supports *M. pumatensis* as a new species.

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Lepiota (Agaricales) in northern Thailand – 1. *L.* section *Stenosporae*

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Abstract —Eleven species of *Lepiota* section *Stenosporae,* reported as new for Thailand, are fully described and illustrated. Five are compared with European and North American collections based on nrITS sequence data. Two species possess a cutis-like pileipellis (*L. aureofulvella, L.* sp. 2) and nine are characterized by a trichodermium (*L. alopochroa, L. castanea, L. citrophylla, L. erythrosticta, L. griseovirens, L. infelix, L. papillata, L. poliochloodes*, *L.* sp. 1). New to science are *Lepiota aureofulvella* (close to *L. boudieri*) and *L. papillata*, which is characterized by small basidiomata covered with gray-brown to olive brown squamules, an orange-white to brownish-orange context, spurred basidiospores with straight or outgrown base, clavate to cylindrical cheilocystidia, and a trichodermal pileus covering. A key to *Lepiota* sect. *Stenosporae* in northern Thailand is provided.

Key words — *Agaricaceae,* biodiversity, taxonomy, saprotroph

Introduction

Members of the genus *Lepiota* (Pers.) Gray (*Agaricaceae*) are in general saprotrophic forest floor dwellers (Singer 1986, Vellinga 2004b). They occur worldwide in tropical and temperate regions, with only a few species reported from desert and arctic-alpine areas (Vellinga 2004b). Sequence analyses of nrITS and LSU data in the *Agaricaceae* have shown that *Lepiota* forms a monophyletic clade together with *Cystolepiota, Echinoderma, Melanophyllum,* and *Pulverolepiota* (Vellinga 2003ab, 2004a).

Lepiota was traditionally divided on morphological grounds into sections based on spore shape and (secondarily) pileus covering structure. Section *Stenosporae* (J.E. Lange) Kühner, typified by *L. pseudofelina* J.E. Lange, is characterized by spores with a distinct outgrowth (spur) at the base, and a trichodermal or cutis-like pileus covering (e.g., Singer 1986, Bon 1993). Molecular-phylogenetic analyses have since reorganized the morphologically defined sections (Vellinga 2003b). Vellinga (2003b) noted that species with spurred spores and a hymenidermal pileus covering have been found to relate closely to species with ellipsoid spores and a hymenidermal pileus covering, while species with spores that lack a distinct spur, are slender, and have a trichoderm (e.g., *L. cortinarius* J.E. Lange) group with species with fusiform spores. Vellinga (2003b) also reported that species with ellipsoid spores and a trichoderm without basal short elements form a monophyletic clade with *Lepiota* sect. *Stenosporae,* but that three subclades can be recognized — two with spurred spores plus one with ellipsoid spores. Here we focus on the two spurred-spored clades.

Among the few *Lepiota* species previously reported from Thailand are *L. clypeolaria* (Bull.) P. Kumm., *L. cortinarius*, *L. cristata* (Bolton) P. Kumm., and *L. pseudohelveola* Hora (Soytong 1994, Chandrasrikul 1996, Chandrasrikul et al. 2008), none of which belongs to *Lepiota* sect. *Stenosporae*. However, the following species in *Lepiota* sect. *Stenosporae* are known from other parts of tropical Asia: *L. alopochroa*, *L. citrophylla*, *L. erythrosticta*, *L. leontoderes* (Berk. & Broome) Sacc., and *L. pyrrhaes* (Berk. & Broome) Sacc. from Sri Lanka (Pegler 1972, 1986; Petch & Bisby 1950); *L. castanea*, *L. erythrosticta*, and *L. griseovirens* from southern India (Kumar & Manimohan 2009); and *L. aurora* E. Horak, *L. castanea*, *L. crepusculata* E. Horak, *L. erythrosticta*, *L. infelix*, *L. luteocastanea* E. Horak, and *L. squamatula* E. Horak from Papua New Guinea (Horak 1981).

Lepiotaceous fungi have been studied in East Asia in recent years (Ge & Yang 2006; Ge et al. 2010; Liang et al. 2009, 2010b; Vellinga et al. 2011). The present study focuses on *Lepiota* sect. *Stenosporae* in northern Thailand, as part of our ongoing efforts to document the diversity of *Agaricaceae* from this area (Zhao et al. 2010, Vellinga et al. 2011). We used morphological and molecular methods to identify and circumscribe our taxa.

Materials & methods

Collecting and examination methods

Collecting sites in Chiang Mai and Chiang Rai provinces, Thailand, were surveyed during the rainy seasons from April to October, 2007–2010. The sites, located within the coordinates 16°06.16′N 98°29.64′E and 19°48.60′N 99°54.60′E at altitudes of 596–1700 m, comprise different vegetation types. Detailed notes, concerning location, vegetation type, soil and substrate were taken in the field. Most specimens were also photographed in the field. All material is deposited in the herbarium of Mae Fah Luang University

(MFLU). Macromorphological characters were noted in the laboratory based on the fresh materials, using standard procedures and terms (Vellinga & Noordeloos 2001). Color annotations in the macroscopical descriptions are from Kornerup & Wanscher (1978), or if preceded by 'Mu.' from Munsell (1975). Microscopical characters were studied from dry specimens and drawn using a drawing tube attached to an Olympus CX-41research compound microscope. Characters were observed in Congo red in ammonia, water, or in 2.5–10% of KOH. Chemical reactions in Melzer's reagent, Cotton blue and Cresyl blue were noted. At least 20 spores per collection were measured in side view. The notation [180,7,6] indicates that measurements were made on 180 spores in seven samples in six collections. The following abbreviations are used: 'L' for lamellae, 'l' for lamellulae, 'avl' for average length, 'avw' for average width, 'Q' for quotient of length and width and 'avQ' for average quotient.

Molecular and phylogenetic methods

DNA EXTRACTION—DNA was extracted from herbarium collections (TABLE 1) according to the instructions of the Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Ltd., Hangzhou, P.R. China).

TABLE 1. *Lepiota* species for the ITS sequences used in the phylogenetic analyses in this study.

SPECIES	COUNTRY	COLLECTION & HERBARIUM	ITS GENBANK ACCESSION NUMBER
L. alopochroa	Thailand	MFLU090178 (MFLU)	HQ647294***
L. andegavensis	France	P.D.H. Roux 2121 (herb. Roux)	AY176461*
L. aureofulvella	Thailand	MFLU090183	HQ647293***
L. boudieri	The Netherlands	E.C. Vellinga 1180 (L)	AF391025*
	Italy	MCVE: 474	FJ998388*
L. cf. *boudieri*	U.S.A	E.C. Vellinga 2601 (UC)	AY176479**
L. castanea	The Netherlands	N.J. Dam 97020 (herb. Dam)	AY176463*
	The Netherlands	H.A. Huijser (herb. Huijser)	AF391026*
L. cingulum	Germany	M. Enderle (L)	AY176359*
L. citrophylla	Thailand	MFLU090172	HQ647295***
L. cristata	U.S.A	P.B. Matheny 1958 (WTU)	AF391051**
L. grangei	Belgium	H.A. Huijser (herb. Huijser)	AY176471*
	Italy	MCVE:4666	FJ998399*
L. griseovirens	Italy	MCVE:13747	FJ998403*
L. ignicolor	The Netherlands	H.A. Huijser (herb. Huijser)	AY176472*
L. cf. *ignipes*	Italy	MCVE: 480	FJ998390**
L. pilodes	The Netherlands	H.A. Huijser (herb. Huijser)	AY176476*
	U.S.A	E.C. Vellinga 3234 (UC)	EF080865**
L. poliochloodes	Thailand	MFLU081272	HQ647296***
L. pseudofelina	Italy	MCVE: 3553	FJ998398*
L. rhodophylla	U.S.A	E.C. Vellinga 2610 (UC)	AY176480**
	U.S.A	E.C. Vellinga 3026 (UC)	EF080864**
L. subalba	The Netherlands	E.C. Vellinga 2242 (L)	AY176489*
L. tomentella	The Netherlands	H.A.Huijser (L)	EF080868*
Lepiota sp.	Thailand	E.C. Vellinga 3881 (MFLU)	HQ647297***
	U.S.A	E.C. Vellinga 3327 (UC)	EF080867**
	U.S.A	E.C. Vellinga 2574 (UC)	AY176484**
	U.S.A	E.C. Vellinga 2603 (UCB)	AY176481**
	U.S.A	E.C. Vellinga 3014 (UC)	EF080866**

* = sequence from Europe, ** = sequence from U.S.A, *** = new sequence from Thailand

PCR AND SEQUENCING—Primers ITS1-F, ITS 1, and ITS4 were used for the nrITS1, 5.8S and nrITS2 regions, and PCR conditions followed Gardes & Bruns (1993). PCR amplified products were cleaned up by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd.). Sequencing was performed using Big Dye chemistry, with the same primers as for PCR, and an ABI PRISM 3100 Genetic Analyzer (both from Applied Biosystems, Foster City, CA, USA). All new sequences were deposited in GenBank.

DATA ANALYSIS—Sequences were edited and contigs assembled using Sequencher 4.2.2 (Gene Codes Corporation, Ann Arbor, MI, USA). The sequences of all taxa belonging to *Lepiota* sect. *Stenosporae* present in GenBank were downloaded, and the complete data set aligned using MAFFT (Katoh et al. 2002, Katoh & Toh 2008) and minimally manually adjusted. Heuristic search of ITS was performed using the maximum parsimony (MP) option of the program PAUP* 4.0 b10 (Swofford 2004), using 1000 heuristic searches, employing TBR branch swapping and random sequence addition. Other setting were as follows: gaps were treated as missing data; all characters are of type unordered and equally weighted; multistate taxa interpreted as uncertainty; starting trees were obtained via stepwise addition; one tree was held at each step during stepwise addition; the steepest descent option was not in effect, branches were collapsed (creating polytomies) if minimum branch length was zero, and MulTrees option was in effect. Bootstrap supports were evaluated using 1000 bootstrap replicates with 10 heuristic searches per replicate, random sequence addition and TBR branch swapping. A Maximum Likelihood analysis was performed with the on line program RAxML (Stamatakis et al. 2008). All free model parameters were estimated by RAxML using a general time-reversible (GTR) substitution matrix and a proportion of invariable sites estimate. One hundred rapid ML bootstraps were performed. The ML tree was visualized with the program Figtree v. 1.3.1 (Rambaut 2009).

Results

Nine named and two unnamed species of *Lepiota* sect. *Stenosporae* were recognized in this study. A key to the eleven taxa is provided and each taxon is provided with a full description, colored photographs (FIGURE 1), and illustrations. TABLE 2 summarizes the morphological characters of the Thai species. Two species (*L. aureofulvella, L.* sp. 2) have a cutis pileus covering and most (except *L. papillata*) have dextrinoid spores.

ITS sequence data for five species from Thailand were compared with those from 23 European and U.S.A. specimens; *Lepiota cristata* with spurred spores and a hymeniform pileus covering is used as outgroup (TABLE 1). The topology of a tree based on Maximum Likelihood analysis is identical to the one presented here (FIGURE 2) and two clades are present in the tree. Clade 1 comprises species with spurred spores and a trichodermal pileus covering; the Thai species *L. citrophylla* and *L. poliochloodes* group together and are sister to *L. grangei, L. griseovirens,* and *L. pseudofelina* from Europe with low bootstrap support; *L. alopochroa* groups with *L. castanea* and forms a clade with *L.* cf. *ignipes* (from North America) with high bootstrap support. Clade 2, comprising species

FIGURE 1. Basidiomata of species of *Lepiota* section *Stenosporae* in the field: a. *L. aureofulvella;* b. *L. citrophylla;* c. *L. castanea;* d. *L. alopochroa;* e. *L. poliochloodes;* f. *L. infelix;* g. *L. papillata;* h. *L.* sp. 2; i. *L. griseovirens;* j. *L. erythrosticta;* k. *L.* sp.1.

with spurred spores and a cutis-like pileus covering, includes *L. andegavensis* and *L. boudieri* (Europe), *L.* cf. *boudieri* and *L. rhodophylla* (U.S.A.), and *L. aureofulvella* (Thailand); *L. aureofulvella* groups with *L. boudieri* with low bootstrap support (FIGURE 2).

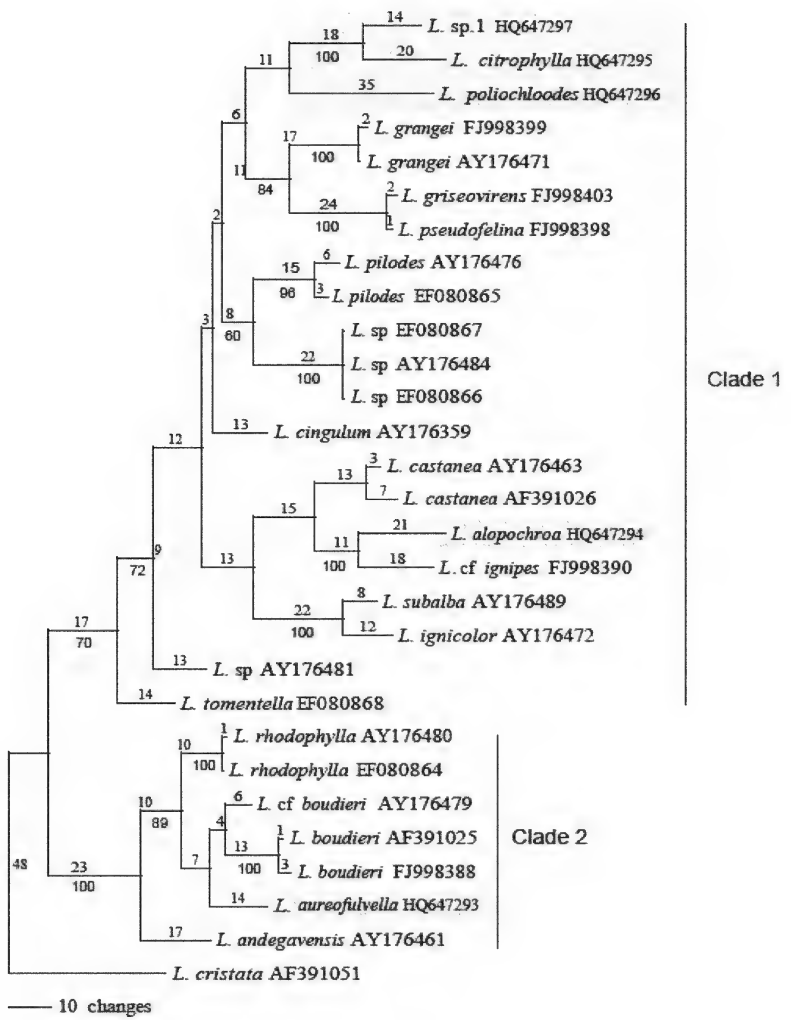

FIGURE 2. Phylogenetic relationships of *Lepiota* section *Stenosporae* based on a Maximum Parsimony analysis of nrITS sequences. Bootstrap values ≥60 % are indicated below the branches. GenBank accession numbers are given for each collection. The topology of a tree based on Maximum Likelihood analysis is identical. Two clades are present in the tree, clade 1 is composed of species with spurred spores and a trichodermal pileus covering; clade 2 is composed of species with spurred spores and a cutis-like pileus covering. *Lepiota cristata* with spurred spores and a hymeniform pileus covering is used as outgroup.

Table 2. Selected *Lepiota* basidiospore and pileus covering structure characters in collections from Thailand.*

Species	Spore size (µm)	avl × avw	Q	avQ	dext[1]	pc[2]
L. alopochroa	7.5–9.3 × 3.8–4.0	8.5 × 3.9	2.0–2.3	2.2	+	Trichoderm
L. aureofulva	5.8–7.5 × 3.0–4.8	7.2 × 3.8	1.6–2.1	1.9	+	Cutis
L. castanea	9.5–13.0 × 3.8–5.2	11.5 × 4.5	2.4–2.6	2.5	+	Trichoderm
L. citrophylla	6.0–8.5 × 3.8–4.3	7.2 × 4.0	1.5–2.0	1.8	+	Trichoderm
L. erythrosticta	7.8–11.3 × 3.8–4.8	9.8 × 4.2	2.2–2.8	2.3	+	Trichoderm
L. griseovirens	6.2–7.0 × 3.5–4.2	6.7 × 3.8	1.5–1.8	1.7	+	Trichoderm
L. infelix	7.5–9.0 × 4.0–4.2	8.3 × 4.0	1.7–2.2	2.0	+	Trichoderm
L. papillata	7.2–11.8 × 4.0–4.8	8.2 × 4.1	1.8–2.5	2.0	–	Trichoderm
L. poliochloodes	6.5–8.2 × 3.0–3.8	7.2 × 3.5	1.6–2.4	2.0	+	Trichoderm
Lepiota sp. 1	7.1–8.4 × 2.8–4.2	7.8 × 3.2	1.9–2.8	2.4	+	Trichoderm
Lepiota sp. 2	6.2–8.0 × 3.2–4.0	7.3 × 3.7	1.6–2.5	1.9	+	Cutis

* No taxa have metachromatic basidiospores; all have cheilocystidia.
[1] dext = dextrinoid, [2] pc = pileus covering.

Taxonomy

Key to species of *Lepiota* section *Stenosporae* identified in this study

1. Pileus covering a cutis made up of cylindrical elements 2
1. Pileus covering a trichoderm made up of erect cylindrical to narrowly clavate elements 3
2. Pileus covering yellowish brown, golden-brown to brown; pileus covering elements cylindrical, septate and not branched ..... *L. aureofulvella*
2. Pileus covering grayish yellow, grayish brown, or slightly reddish brown; pileus covering elements cylindrical, septate and sometimes branched ... *L.* sp. 2
3. Basidiomata pale yellow to light yellow ..... *L. citrophylla*
3. Basidiomata not yellow 4
4. Basidiomata pinkish; pileus with reddish white to pinkish squamules ... *L. erythrosticta*
4. Basidiomata not pinkish; pileus without reddish white to pinkish squamules, but with gray, brown, greenish to dark colors 5
5. Pileus covered with gray to grayish brown, dark gray or olive brown squamules ... 6
5. Pileus covered with brown to dark brown or reddish brown squamules 8
6. Pileus umbonate with high umbo or papilla, covered with brownish gray to olive brown squamules ..... *L. papillata*
6. Pileus umbonate with wide umbo, covered with gray to grayish brown, sometimes olivaceous squamules 7
7. Pileus with gray to grayish brown small plush-like squamules or floccules; smell mild; basidiospores 6.5–8.2 × 3.0–3.8 µm ..... *L. poliochloodes*
7. Pileus with small plush-like squamules or floccules, grayish brown to black, sometimes with a hue of green to olive-brown; smell fruity; basidiospores 6.2–7.0 × 3.5–4.2 µm ..... *L. griseovirens*

8. Pileus covered with reddish brown small scales or squamules. . . . . *L.* sp. 1
8. Pileus covered with light brown to dark brown squamules. 9

9. Pileus convex without or with low umbo. . . . . *L. infelix*
9. Pileus umbonate or with distinct umbo. 10

10. Pileus covered with dark brown to dark at center (umbo), with brown squamules around center toward margin; smell spicy; basidiospores 9.5–13 × 3.8–5.2 µm . . . . . *L. castanea*
10. Pileus umbonate, covered with brown squamules toward margin; smell fruity; basidiospores 7.5–9.3 × 3.8–4.0 µm . . . . . *L. alopochroa*

Lepiota alopochroa (Berk. & Broome) Sacc., Syll. Fung. 5: 63. 1887. FIG. 3

≡ *Agaricus alopochrous* Berk. & Broome, J. Linn. Soc., Bot. 11: 510. 1871.

≡ *Lepiotula alopochroa* (Berk. & Broome) E. Horak, N.Z. J. Bot. 18: 185. 1980.

PILEUS 14 mm, umbonate, expanding to plano-concave with low umbo, with inflexed margin, brown (7E6–7) at umbo, rough or with crowded squamules, with surface breaking up around umbo, with brown (7E6–7) fibrillose squamules toward margin, on light brown (6D5–6) fibrillose background, with peeling surface at margin and white background, with white fibrillose remnants of partial veil and margin exceeding lamellae. LAMELLAE free, crowded, ventricose, 1 mm wide, white or pale yellow to orange-white (4A3, 5A2), with white serrulate edge. STIPE 35 × 2.5–4 mm, cylindrical or slightly tapering to apex and wider at base, with grayish orange to brownish orange (5B4–5, 6C5) background, white fibrillose at annular zone, with brown (7E6–7) squamules or fibrillose squamules at middle zone downward base, with white rhizomorphs at base, hollow. ANNULUS as an annular zone. CONTEXT in pileus white, 0.5 mm wide; in stipe concolorous with surface. SMELL fruity. TASTE unknown. SPORE PRINT white.

BASIDIOSPORES [25,1,1] 7.5–9.3 × 3.8–4.0 µm, avl × avw = 8.5 × 3.9 µm, Q = 2.0–2.3, avQ = 2.2, in side view oblong to cylindrical, spurred, truncate or with outgrown base, with acute apex, in frontal view fusiform to oblong, thick-walled, hyaline, dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl blue. BASIDIA 13–17 × 6.0–8.0 µm, clavate, narrowly clavate, rarely subclavate, 4-spored, thick-walled, hyaline. LAMELLA EDGE sterile. CHEILOCYSTIDIA 25–37 × 5–7 µm, narrowly clavate to cylindrical, with rounded apex, rarely utriform, thin-walled, hyaline. PLEUROCYSTIDIA absent. PILEUS COVERING a trichoderm made up of narrowly clavate to cylindrical elements, 50–113 × 3.5–18 µm, brown and thick-walled, with parietal pale brown pigment; underlayer with hyaline to pale brown hyphae, 2.5–4 µm wide. CLAMP CONNECTIONS present in all tissues.

HABITAT AND DISTRIBUTION—Solitary, saprotrophic and terrestrial on soil, on dead leaves in bamboo forest. Known from one locality in northern Thailand.

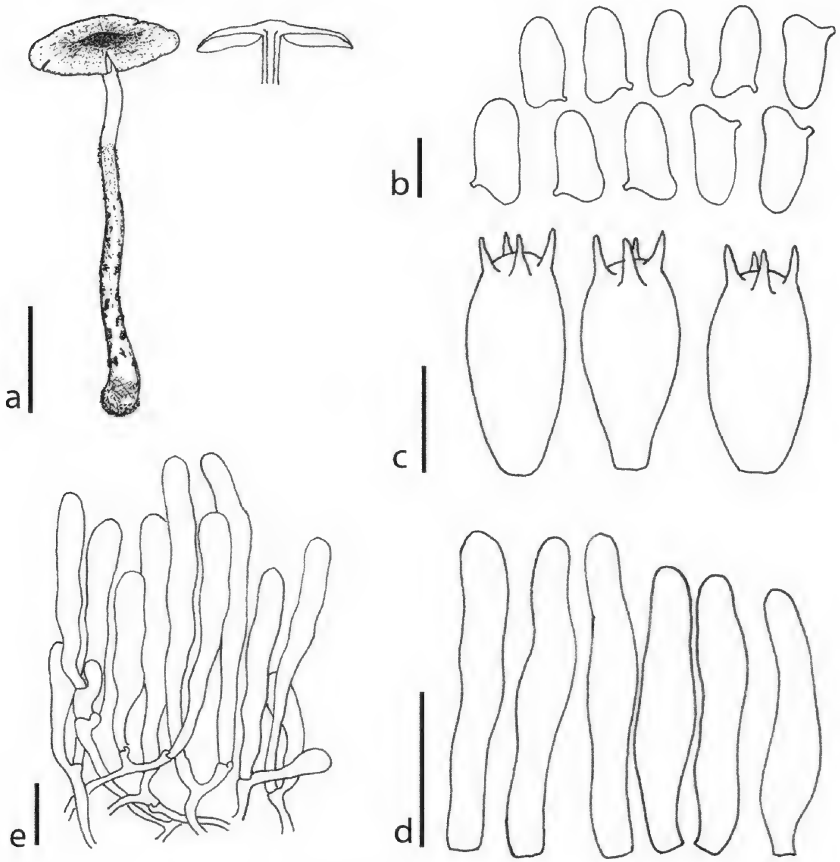

Figure 3. *Lepiota alopochroa* (MFLU090178).
a. basidiomata; b. basidiospores; c. basidia; d. cheilocystidia; e. pileus covering.
Scale bars: a = 10 mm; b–d = 10 µm; e = 20 µm.

Material examined: **THAILAND**, **Chiang Mai Prov.**, Mae Taeng Distr., Mok Fah Waterfall, 20°02′43.1″N, 99°52′35.0″E, 596 m alt., 6.VIII.2008, P. Sysouphanthong, MFLU090178.

Discussion—As a representative of the *Lepiota castanea* group, *L. alopochroa* is characterized by relatively short spores (averaging 8.5 × 3.9 µm) and relatively short elements in the pileus covering; the cheilocystidia are narrowly clavate to cylindrical. Despite these short spores and short pileus covering elements it differs from *L. ignicolor* Bres. in nrITS sequences (Figure 2). The Thai collections of *L. castanea* have darker basidiomata, longer spores, and longer elements in the pileus covering.

Although the collection reasonably well fits *L. alopochroa* as described by Pegler (1972, 1986) and Horak (1980, as *Lepiotula alopochroa*), there are no available molecular data to support this conclusion.

Lepiota aureofulvella Sysouphanthong, K. D. Hyde, Chukeatirote & Vellinga, **sp. nov.** FIG. 4

MYCOBANK MB 519671

Pileus 15–40 mm, convexus vel campanulatus, umbonatus, plano-concavus, fibrillosus, flavidus-brunneus vel auratus. Lamellae liberae, albae vel pallide luteae, 5-6 mm. Stipes 25–45 × 2.5–5 mm, cylindricus vel subclavatus, fibrillosus, albus dein laete brunneus. Basidiosporae 5.8–7.5 × 3.0–4.8 µm, calcaratae. Basidia 16. –21.5 × 5.5–6.3 µm, clavata. Cheilocystidia 17.5–27.5 × 5–10 µm, clavata, anguste clavata. Epicutis ex cellulis cylindraceis vel anguste clavatis. Fibulae adsunt.

HOLOTYPUS: Thailand, Chiang Mai Prov., Mae Taeng Distr., Mok Fah Waterfall National Park, 20°02′43.1″N, 99°52′35.0″E, 596 m. alt., 8.VIII.2008, P. Sysouphanthong PS110 (MFLU090183, **holotype**).

ETYMOLOGY: '*aureofulvella*' means golden-brown and refers to a close relationship with *L. boudieri* (syn. *L. fulvella* Rea)

PILEUS 15–40 mm, convex to umbonate, expanding to plane or plano-concave, with straight margin, with crowded brown (6E7–8) fibrils at umbo, with concentrically crowded fibrillose squamules from umbo toward margin, yellowish brown to golden brown (5D5–7), on white background, when mature surface peeling from background; margin fringed and exceeding lamellae. LAMELLAE free, crowded, ventricose, 5–6 mm wide, white, with white serrulate edge. STIPE 25–45 × 2.5–5 mm, cylindrical to subclavate, with white to light brown (6D4–5) background, white fibrillose in upper zone, at base with slightly brown (6E4–8) fibrillose squamules, with white rhizomorphs at base, hollow. ANNULUS an annular zone, with white fibrils. CONTEXT in pileus white and 4.5–5 mm wide; in stipe concolorous with surface. SMELL fruity. TASTE unknown. SPORE PRINT white.

BASIDIOSPORES [60,3,3] 5.8–7.5 × 3.0–4.8 µm, avl × avw = 7.2 × 3.8 µm, Q = 1.6–2.1, Qav = 1.9, cylindrical to oblong, with truncate to spurred base, triangular or with curved abaxial side, in frontal view cylindrical, thick-walled, hyaline, dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl blue. BASIDIA 16.3–21.5 × 5.5–6.3 µm, clavate, slightly thick-walled, 4-spored. Lamella edge sterile. CHEILOCYSTIDIA 17.5–27.5 × 5–10 µm, clavate, narrowly clavate, utriform, cylindrical, hyaline, slightly thick-walled. PLEUROCYSTIDIA absent. PILEUS COVERING a cutis made up of cylindrical elements, sometimes with narrowly clavate terminal elements, 40–130 × 5.5–14 µm, thick-walled, with brown parietal pigment; underlayer with hyaline to pale brown hyphae, 3–4 septate, 5.0–13 µm wide. CLAMP CONNECTIONS present in all tissues.

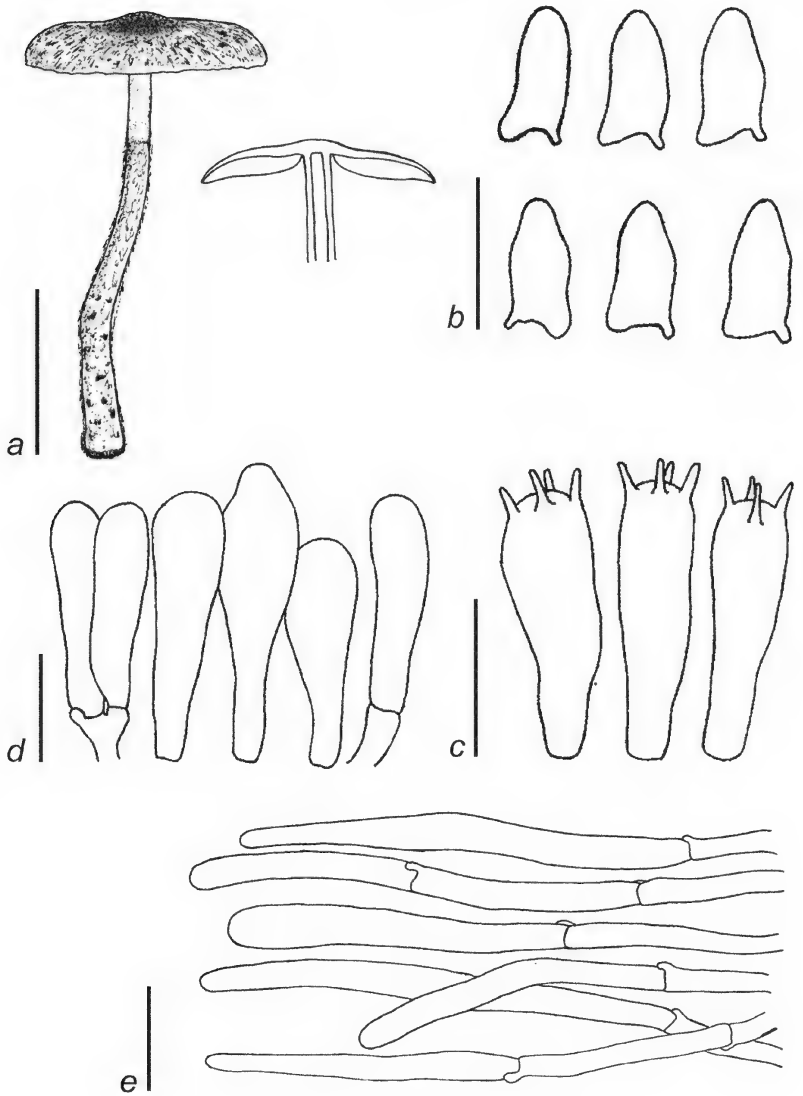

Figure 4. *Lepiota aureofulvella* (MFLU090183).
a. basidioma and a section; b. basidiospores; c. basidia;
d. cheilocystidia; e. elements of pileus covering.
Scale bars: a = 10 mm, b–e =10 µm.

Habitat and distribution—growing in a small to large group; saprotrophic on decayed wood, soil rich in humus, and found in forest with dominant bamboo; in one locality in northern Thailand.

Additional material examined: **THAILAND**, **Chiang Mai Prov.**, Mae Taeng Distr., Mok Fah Waterfall National Park, 20°02′43.1″N, 99°52′35.0″E, 596 m. alt., 24.VI.2010, P. Sysouphanthong, MFLU100592; ibidem, 9.VI.2010, P. Sysouphanthong, MFLU100621.

Discussion—*Lepiota aureofulvella* is characterized by the yellowish brown or light brown to golden brown color of the pileus covering, clavate to utriform cheilocystidia, and cutis-like pileus covering.

The only similar species, *L. alopochroa,* resembles *L. aureofulvella* in color and general stature but differs in the trichodermal pileus covering.

Lepiota squamatula from Papua New Guinea, which is discussed and illustrated as having a cutis-like pileus covering but diagnosed in Latin as having a trichoderm, differs in having orange-brown squamules and fibrils on the pileus, longer (8.0–9.0 µm) and narrower (2.5–3.5 µm) basidiospores, and a pileus covering "composed of cylindric interwoven hyphae forming a cutis or trichoderm" (Horak 1980).

Some north temperate species with a cutis-like pileus covering and spurred basidiospores are similar to *L. aureofulvella. Lepiota boudieri* Bres. is similar in morphology but has somewhat darker and less golden colors and shorter (5.8–7.5 µm) basidiospores (Vellinga 2001); the western North American *L. rhodophylla* Vellinga is distinguished by a pinkish-brownish pileus covering with a pinkish brown margin and pink to pale brownish lamellae (Vellinga 2006); and the French species, *L. andegavensis* Mornand (Mornand 1983), has very dark pileus colors.

The nrITS based phylogeny (Figure 2) clearly separates *L. aureofulvella* from all the other species in clade 2 with a cutis-like pileus covering and for which sequence data are available.

Lepiota castanea Quél., C.r. Ass. Franç. Av. Sci. (Reims 1880) 9: 661. 1881. Fig. 5

Pileus 15–20 mm, umbonate to campanulate with wide umbo, with inflexed margin, with crowded fibrils at umbo, brown (7E6–8) and changing to dark brown to dark when dry (7F6–8), with rough umbo, with rough surface around umbo to margin, brown (6E5–6) and paler at margin (6D5–7), with surface soon breaking up and becoming squamules or patch-like squames with upcurved tips, on white background, at margin white fibrillose and with light brown fibrillose squamules. Lamellae free, crowded, ventricose, 3 mm wide, white, with smooth edge. Stipe 40–58 × 1.8–3 mm, cylindrical or slightly tapering to apex, white from apex to 1/3 of length, white or pale orange to light orange from annular zone to base with squamule-like, light brown to brown (6D5–7, 6E6) patches and bands, hollow, with white rhizomorphs at base. Annulus an annular zone, with squamules or fibrillose partial veil. Context in pileus white and dull, 2–2.5 mm wide, in stipe white. Smell spicy. Taste unknown. Spore print white.

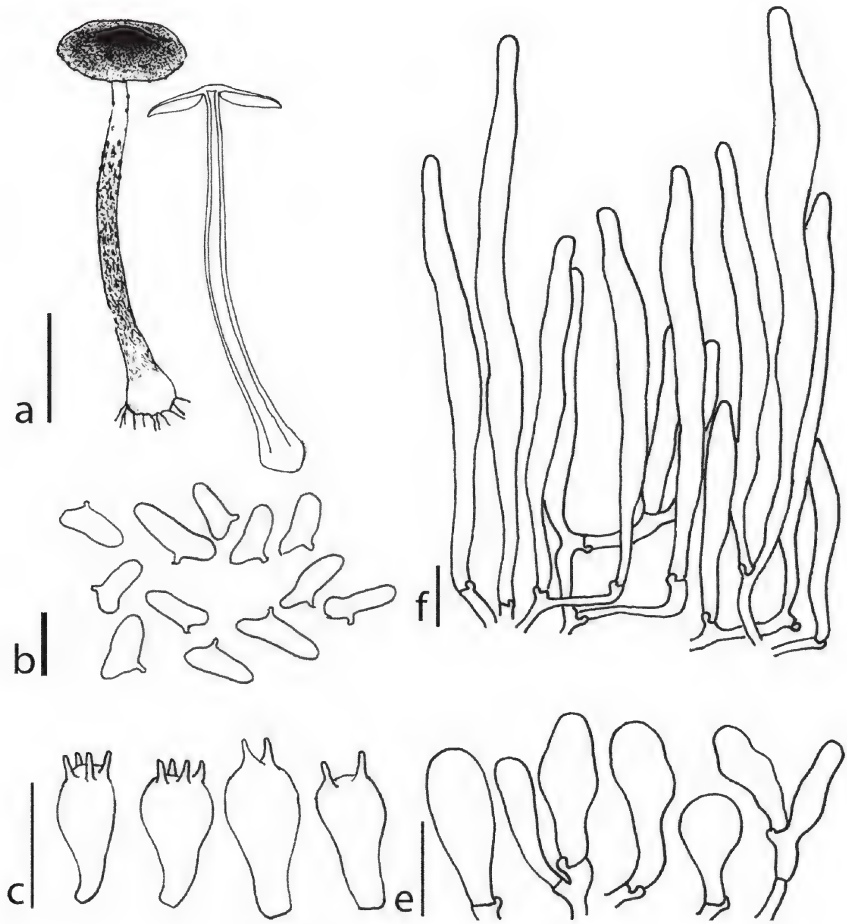

Figure 5. *Lepiota castanea* (MFLU090106).
a. basidioma and a section; b. basidiospores; c. basidia; d. cheilocystidia; e. pileus covering.
Scale bars: a = 10 mm; b–e = 10 µm..

Basidiospores [75,2,2] in side view 9.5–13 × 3.8–5.2 µm, avl × avw = 11.5 × 4.5 µm, Q = 2.4–2.6, Qav = 2.5, in side view cylindrical, with truncate to distinctly spurred base, mostly with outgrown broad basal spur and long hilar appendage, some with a straight base, in frontal view oblong to cylindrical, hyaline, slightly thick-walled, dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl blue. Basidia 18–23 × 9.0–10 µm, clavate, 4-spored, with or without mucilaginous contents. Lamella edge sterile. Cheilocystidia

18–36 × 5.5–12.5 µm, usually clavate to narrowly clavate, with acute or rounded apex, often utriform, thin-walled. Pleurocystidia absent. Pileus covering and stipe covering a trichoderm made up of narrowly clavate to cylindrical elements, wider at middle and narrowing to apex and base, 40–375 × 6.5–35 µm, with slightly thickened walls, pale brown to brown-walled, with parietal brown pigment, with rare short clavate elements; under layer with cylindrical hyphae, hyaline to pale brown-walled, 2.5–5 µm wide. Clamp connections present in all tissues.

Habitat and distribution—growing solitary or in a small group, saprotrophic and terrestrial on forest floor with high amount of humus, only found in deciduous forest at high elevation (Doi Inthanon National Park, Chiang Mai Province, Thailand). Widely distributed in Europe and North America and temperate parts of Asia (Vellinga 2001), also recorded from India and Nepal (Manandhar & Adhikari 1994), and from Papua New Guinea (Horak 1981). This is a new record for Thailand.

Material examined: **THAILAND, Chiang Mai Prov.**, Chom Thong Distr., Doi Inthanon National Park, Junction of Highway 1009 and Road to Mae Cheam, 19°31.58′N, 98°29.64′E, 1700 m alt., 20.VI.2008, P. Sysouphanthong MFLU090106; 15.VII.2008, P. Sysouphanthong, MFLU090154.

Discussion—The *L. castanea* complex is in need of a combined morphological-molecular approach to resolve the species borders. Vellinga (2001), who took a pragmatic view, considered *L. castanea* a very variable species, variable in terms of spore length and length of pileus covering elements. Migliozzi & Zecchin (1997, 2000) recognized several species in the complex: *L. rufidula* Bres. for the long-spored taxon, commonly (also by us) named *L. castanea*; *L. ignipes* Bon is considered a synonym of *L. rufidula*; *L. castanea* s. str. for one short-spored species and *L. ignicolor* for a taxon with small basidiomata and spores tending toward ovoid. A collection referred to *L. ignicolor* is sister to *L. subalba* P.D. Orton in the phylogenetic analyses based on nrITS sequences (Figure 2); *L. subalba* has short elements in the pileus covering but whitish basidiomata. The characters of the Italian collection named *L. ignipes* in GenBank (accession number FJ998390) are not known, but the molecular data indicates that it differs from the other taxa in this complex.

Lepiota cingulum Kelderman, which resembles *L. castanea*, has predominantly pinkish brown basidiomata and a pileus covering with terminal elements that tend to taper towards the tips (Kelderman 1994).

The record of *L. castanea* from Nepal (Manandhar & Adhikari 1994–95) probably refers to another species, as the spores are described as ellipsoid.

Lepiota castanea is rare in northern Thailand and was found only at one locality at 1700 m asl on Thailand's highest mountain early in the rainy season. It represents the temperate Eurasian element of the Thai *Lepiota* mycota.

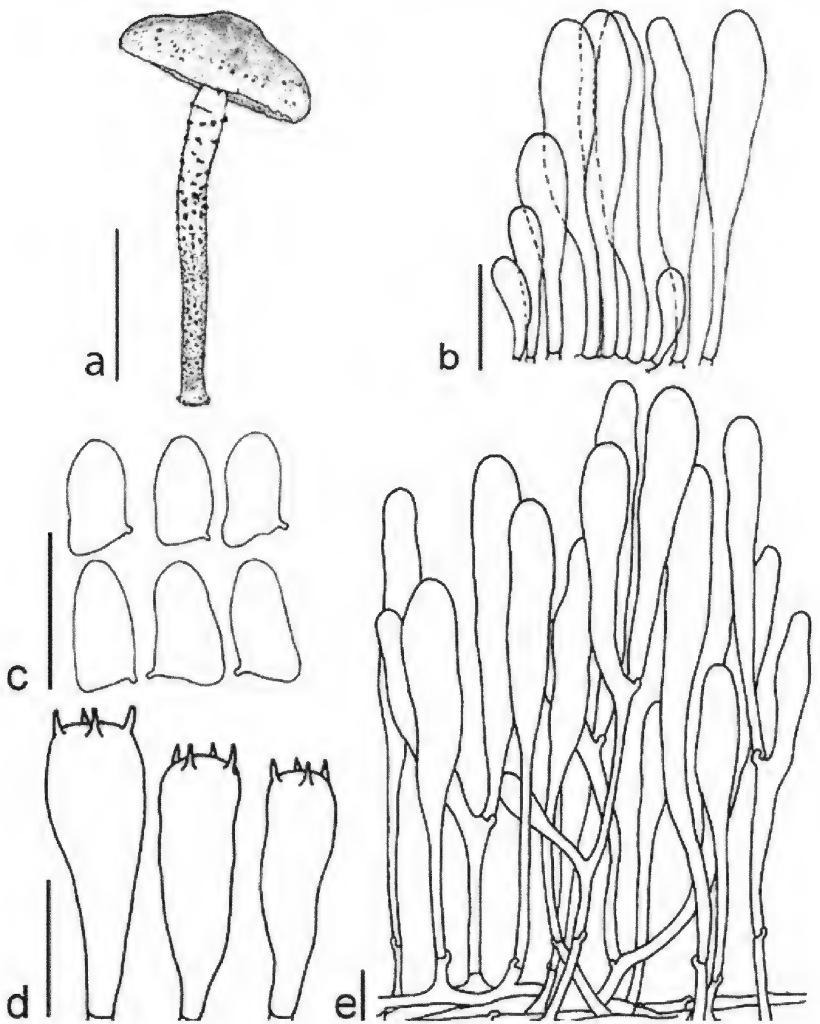

FIGURE 6. *Lepiota citrophylla* (MFLU090101).
a. basidiomata; b. cheilocystidia; c. basidiospores; d. basidia; e. pileus and stipe covering.
Scale bars a = 10 mm; b–e = 10 µm.

Lepiota citrophylla (Berk. & Broome) Sacc., Syll. Fung. 5: 57. 1887. FIG. 6

≡ *Agaricus citrophyllus* Berk. & Broome, J. Linn. Soc., Bot. 11: 509. 1871.

EXCLUDED: *Lepiota citrophylla* sensu Rea (1922), Akers & Sundberg (1998), Liang et al. (2010a).

PILEUS 15–35 mm, subglobose to conical, expanding to campanulate, with moderately wide umbo, with inflexed margin, at umbo covered with fine

squamules to floccules, brown (6D7) at umbo and with light brown to yellowish brown (5D6–8) squamules toward margin on pale yellow to light yellow (4A3–4) background; margin with distant squamules, sometimes with partial veil. Lamellae free, crowded, ventricose, 2.5–3.8 mm wide, pale yellow to light yellow (3A4–4A4), with concolorous serrulate edge. Stipe 60–65 × 2–3 mm, cylindrical, slightly wider at annular zone; surface covered with squamules from annular zone to middle and with small light brown to brown (5D6–6D7) squamules at middle to base, on light yellow (4D4) background; hollow. Annulus an annular zone or made up of squamules. Context in pileus 1.8–2 mm wide and pale yellow (3A3), in stipe light yellow (4D4). Smell as burnt rubber. Taste unknown. Spore print white.

Basidiospores [100, 5, 5] in side view 6.0–8.5 × 3.8–4.3 µm, avl × avw = 7.2 × 4.0 µm, Q = 1.5–2.0, avQ = 1.8, in side view with straight or outgrown spur at base, ellipsoidal to cylindrical with rounded or more acute apex, in frontal view oval, cylindrical, hyaline, thick-walled, strongly dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl Blue. Basidia 19–37 × 6.5–8.5 µm, clavate to slightly narrowly clavate, 4-spored, hyaline, with thin wall. Lamella edge sterile. Cheilocystidia 15–60 × 5.0–10 µm, narrowly clavate, sometimes clavate, utriform, rarely narrowly fusiform, or wider at center. Pleurocystidia absent. Pileus covering a trichoderm made up of erect narrowly clavate elements, often narrowed into pedicel, 50–160 × 8.5–17 µm, with thin brown wall, with parietal and intracellular brown pigment, with an under layer of cylindrical hyphae, 2–6.5 µm wide, with thin hyaline to slightly pale brown walls, with intracellular pigment. Stipe covering with same structure as pileus covering. Clamp connections present in all tissues.

Habitat and distribution—solitary or in small groups, terrestrial in soil with decaying leaves and wood, found in deciduous forest and pine forests of northern Thailand. Widely distributed in the tropics: Sri Lanka (Pegler 1972), Trinidad (Dennis 1952), Kenya and East Africa (Pegler 1977), and India (Natarajan & Manjula 1983). This is the first record from Thailand.

Material examined: **THAILAND**, **Chiang Mai Prov.**, Mae Taeng Distr., Hot Spring National Park, 16°06′16.1″N, 99°43′07.9″E, 780–805 m alt., 30.VII.2008, P. Sysouphanthong, MFLU090172; 8.VIII.2010, MFLU090185; Mae Sae Village, 19°07′13.7″N, 98°43′52.9″E, 962 m alt., 14.VI.2008, P. Sysouphanthong, MFLU090101; **Chiang Rai Prov.**, Mae Fa Luang Distr., Doi Tung National Park, 10.VI.2009, P. Sysouphanthong, MFLU100405; Wiang Chiang Rung Distr., Hui Mae Sack Waterfall National Park, 26.VIII.2009, P. Sysouphanthong, MFLU100424.

Discussion—This species is found during the whole rainy season and is widespread in northern Thailand. It is quite variable in basidiome size and pileus covering color. *Lepiota citrophylla* is the only species of sect. *Stenosporae* in northern Thailand characterized by predominantly yellow basidiome colors. The Thai material is very similar to collections from Sri Lanka described by

Pegler (1972, 1986), but he did not recover cheilocystidia in the material from Sri Lanka and his described shorter pileus covering elements are shorter (30–80 × 12–17 µm). There can be two reasons for this: the material he studied is young, or the longer elements were degraded, broken, or otherwise not easily found and measured.

The name *L. citrophylla* has been applied to both a European species (e.g. Rea 1922; Kühner 1934) and an American species (Akers & Sundberg 1998), but those have non-spurred spores; the European species was subsequently described as *L. xanthophylla* P.D. Orton (Orton 1960).

Lepiota luteocastanea is very close to *L. citrophylla* in yellow basidiome colors, but L. *luteocastanea* is distinctly purple tinged on the pileus and has fusoid-cylindric elements in the pileus squamules (Horak 1981). *Lepiota subcitrophylla* Hongo (1956) from Japan also comes close to *L. citrophylla* but turns blue when bruised or damaged. *Verrucospora vulgaris* Pegler, which resembles *L. citrophylla* in the brown squamules, yellow tinged basidiomata, and persistent annulus, does not have free lamellae and produces angular basidiospores (Pegler 1977).

Lepiota citrophylla has previously been known only from tropical parts of Asia and is reported from Thailand here for the first time. A recent report of this species from China refers to a different species representing *Lepiota* sect. *Lepiota*, as the spores are not spurred (Liang et al. 2010a).

Lepiota erythrosticta (Berk. & Broome) Sacc., Syll. Fung. 5: 62. 1887. FIG. 7
≡ *Agaricus erythrostictus* Berk. & Broome, J. Linn. Soc., Bot. 11: 508. 1871.

PILEUS 14 mm, parabolic, with straight margin, with crowded reddish or pinkish (8A2–3) squamules at center and toward margin, sometimes with fibrillose squamules, on white to pale red (8A3) background; margin sulcate, with reddish white (8A2–3) fibrillose squamules and partial veil remnants. LAMELLAE free, slightly crowded, broadly ventricose, white to pale orange (6A3), with white floccose edge. STIPE 32 × 2–2.5 mm, cylindrical, with, 3–3.5 mm wide basal bulb, white (6A2) at apex, covered with light brown to reddish or pinkish (6D4, 8A2) fibrillose squamules on orange fibrillose background from annular zone downward, hollow. ANNULUS an annular zone, with fibrils and fibrillose squamules similar to those on pileus. CONTEXT in pileus white, turning orange white to pale orange (5A2–3), 1 mm wide; in stipe orange white (5A2) in apical zone, grayish red to reddish brown (8C5–8D5) from midway downward. TASTE unknown. SMELL unknown. SPORE PRINT white.

BASIDIOSPORES [25,1,1] 7.8–11.3 × 3.8–4.8 µm, on average 9.8 × 4.2 µm, Q = 2.2–2.8, avQ = 2.3, in side-view cylindrical with truncate to spurred base, narrowly triangular, in frontal view cylindrical, thick-walled, hyaline, not dextrinoid, congophilous, cyanophilous, metachromatic in Cresyl blue. BASIDIA

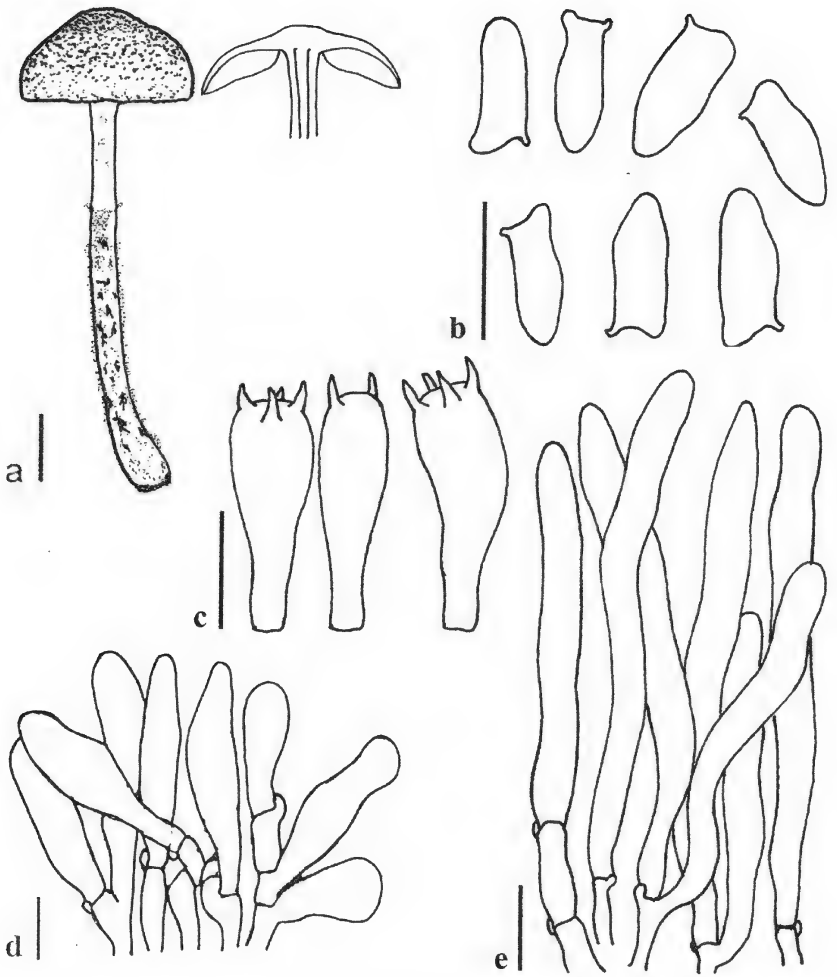

FIGURE 7. *Lepiota erythrosticta* (MFLU090034).
a. basidioma; b. basidiospores; c. basidia; d. cheilocystidia; e. pileus covering.
Scale bars: a = 1 mm; b–d = 10μm; e = 20 μm.

16–28 × 5–8 μm, clavate, 4-spored, rarely 2-spored. LAMELLA EDGE sterile. CHEILOCYSTIDIA 19–32 × 8–13 μm, irregularly clavate or sphaeropedunculate, utriform, often narrowly fusiform, thick-walled, hyaline. PLEUROCYSTIDIA absent. PILEUS COVERING a trichoderm made up of long cylindrical elements, 70–170 × 4.5–12.0 μm, hyaline to pale pink-walled, with parietal and intracellular pink pigment, with underlayer made up of hyaline hyphae, 4–10

μm wide. Stipe covering a trichoderm similar to pileus covering. Clamp connections present in all tissues.

Habitat and distribution—Growing solitary, on humus-rich soil with decayed leaves and wood, in forest with dominant bamboo; found in one locality in northern Thailand. Distributed in Sri Lanka (Pegler 1972), Brazil (Wartchow et al. 2008), and Trinidad (Dennis 1952). This is a new record for Thailand.

Material examined: **THAILAND**, **Chiang Mai Prov.**, Mae Taeng Distr., Hot Spring National Park, 16°06′16.1″N, 99°43′07.9″E, 780–805 m alt., 4.VIII.2007, P. Sysouphanthong, MFLU090034.

Discussion—*Lepiota erythrosticta* is recognized by the reddish or pinkish squamules on pileus and stipe and pinkish lamellae. The non-dextrinoid spores and metachromatic reaction of the spore wall in Cresyl blue distinguishes *L. erythrosticta* from all other species in sect. *Stenosporae.*

The sole Thai collection consisted of young basidiomata. Wartchow et al. (2008) recorded longer pileus and stipe covering elements for material from Brazil; Dennis (1952), who recorded this species from Trinidad, noted smaller basidiospores than those found in material from Thailand. Pegler (1972), who studied the Sri Lankan type material but did not report the reaction of spore walls in Cresyl blue, also cited slightly smaller basidiospores. It is possible that the South American reports represent a different taxon.

This species is rare in Thailand; it was only found once (August 2007) in the middle of rainy season at Hot Spring National Park but not the other three years the survey was conducted. Like *L. citrophylla*, *L. erythrosticta* is a species with a tropical distribution pattern.

Lepiota griseovirens Maire, Bull. trimest. Soc. mycol. Fr. 44: 37. 1928. Fig.8

Misapplied name: *Lepiota pseudofelina* sensu Bon (1993), Candusso & Lanzoni (1990).

Excluded: *Lepiota griseovirens* sensu Reid (1972), Bon (1993).

Pileus 8–13 mm, conico-campanulate, expanding to umbonate, plano-convex with broad umbo, with straight margin, at centre covered with tomentose tufts, grayish brown (7F3, 7E3, 7D3) to black, sometimes with a hue of green to olive-brown (4F4–5), toward margin cracked into small squamules, paler than at center, on orange-white to pale orange (5A2–3) background; margin slightly fringed, exceeding lamellae in mature specimen. Lamellae free, slightly crowded, ventricose, and rounded near stipe, 2 mm wide, orange-white to pale orange (5A2), with white eroded to flocculose edge. Stipe 16–20 × 1.5–2.0 mm, with 2.0–2.5 mm wide base, cylindrical, slightly wider at base, white to orange-white (5A2) innately fibrillose above annular zone and below annular zone downward toward base, with scattered grayish brown (7F3, 7E3, 7D3) to black squamules as on pileus, with brownish-orange (6C4–5) background,

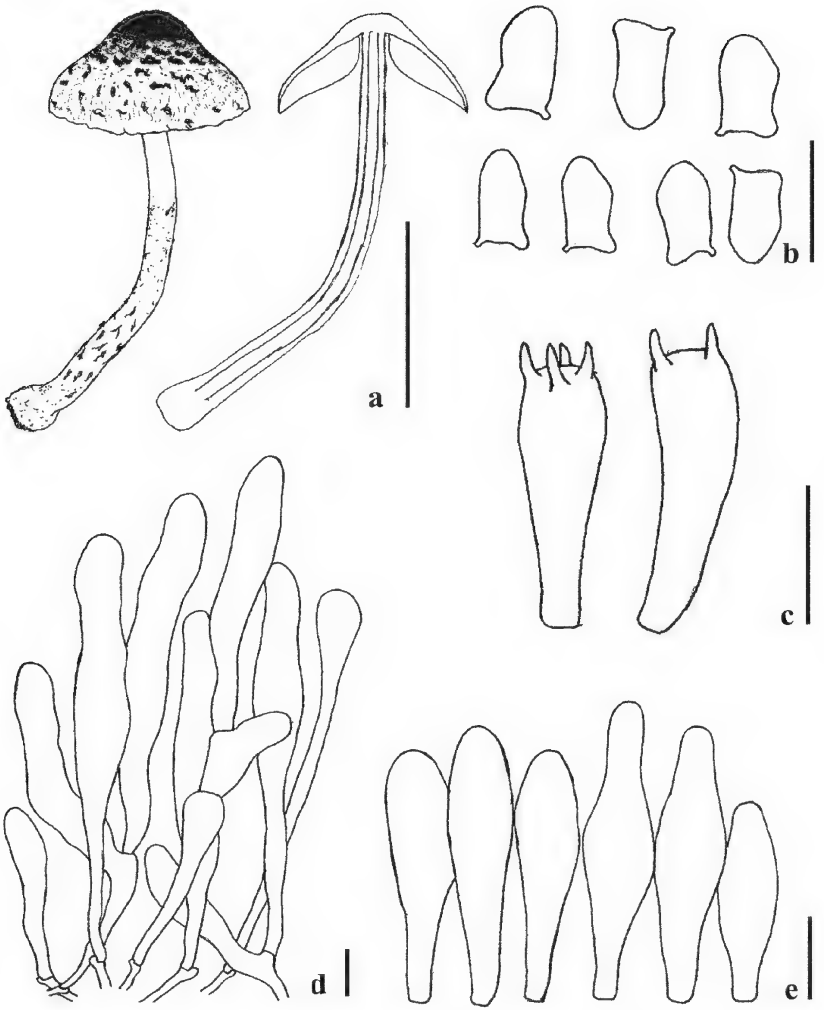

Figure 8. *Lepiota griseovirens* (MFLU100554).
a. basidiomata and a section; b. basidiospores; c. basidia; d. pileus covering; e. cheilocystidia.
Scale bars: a = 10 mm; b–e = 10 µm.

with white mycelium cords, hollow. Context in pileus white, 1.5–2 mm wide, in stipe concolorous with surface. Smell fruity. Taste unknown. Spore print white.

Basidiospores [50,2,1] in side view 6.2–7.0 × 3.5–4.2 µm, avl × avw = 6.7 × 3.8 µm, Q = 1.5–1.8, avQ = 1.7, oblong, with truncate base, with hilar

appendage, thick-walled, strongly dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl blue. Basidia 17–20 × 5.5–7 µm, clavate, 4-spored, often with 2 sterigmata. Lamella edge sterile. Cheilocystidia 22–30 × 6–8 µm, clavate, narrowly clavate, narrowly utriform. Pleurocystidia absent. Pileus covering a trichoderm made up of cylindrical to narrowly fusiform elements, without short clavate elements at base, 40–140 × 7.0–13 µm, thin-walled, with brown parietal pigment; basal hyphae cylindrical and hyaline, 7.5–12.5 µm wide. Stipe covering (squamules) a trichoderm as pileus covering. Clamp connections present in all tissues.

Habitat and distribution—growing in a small group with few basidiomata, terrestrial, and saprotrophic on ground of rain forest with dominant pine. This is the first record for Thailand. Also known from Europe (Vellinga 2001, Bon 1993).

Material examined: **THAILAND, Chiang Rai Prov.**, Mae Fa Luang Distr., Doi Tung National Park, 17.VII.2010, P. Sysouphanthong, MFLU100554.

Discussion—*Lepiota griseovirens* is rare in Thailand and was only found once, in Doi Tung National Park. The Thai collection is characterized by grayish brown to black pileus squamules that sometimes have a green to olive-brown hue. Its spores fall into the lower part of the range recorded for European collections (Vellinga 2001), but all other characters (including shape of the cheilocystidia) fit well with the European species concept.

Vellinga & Huijser (1993) discussed the naming of this species, which was then mainly known as *L. pseudofelina*. The two nrITS collections labeled as *L. pseudofelina* in Figure 2 previously were referred to two different names: *L. pseudofelina* and *L. griseovirens*.

Lepiota infelix E. Horak, Sydowia 33: 133. (1981). Fig. 9

Pileus 19–23 mm, when young convex or with low umbo, expanding to plano-concave with low umbo, with straight or slightly inflexed margin, when young dark brown (7F5–6), soon breaking open into squamules, crowded and dark brown (7F5–6) at centre and umbo, with fibrillose to fibrillose light brown to brown (6D7–8) squamules around centre toward margin, on white fibrillose background; margin split, fringed, exceeding lamellae when mature. Lamellae free, slightly crowded, ventricose, 3 mm wide, white, fragile, with white eroded edge. Stipe 20–25 × 1.2–1.5 mm, cylindrical, slightly wider at base; background white at apical zone, darker down towards base, light brown (6D7) at base, when mature turning grayish orange to brownish orange (6B4, 6C4–6), with brown (6E5–7) squamules from centre down towards base, hollow. Annulus as annular zone, with squamules. Context in pileus white to pale yellow (3A2), 1 mm wide; in stipe concolorous with surface. Smell mild. Taste unknown. Spore print white.

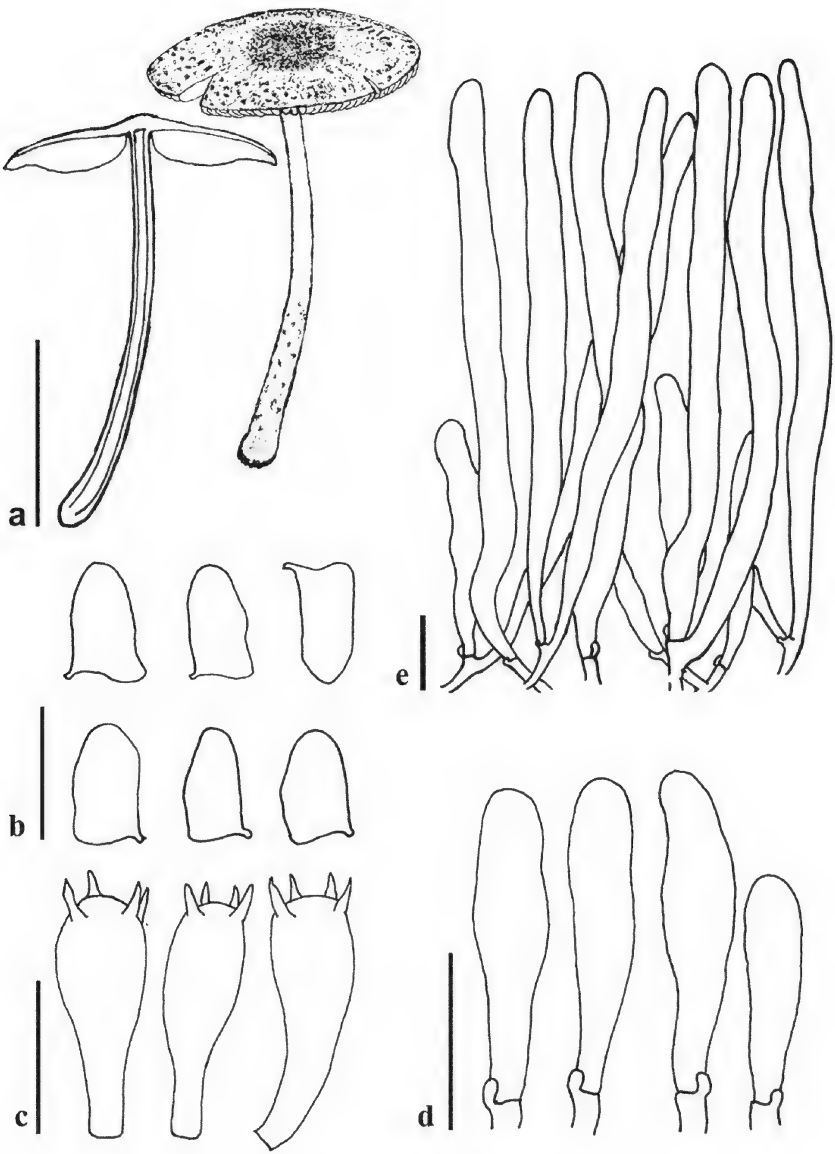

Figure 9. *Lepiota infelix* (MFLU090014).
a. basidioma; b. basidiospores; c. basidia; d. pileus covering; e. cheilocystidia.
Scale bars: a = 20 mm; b–e = 10 μm.

Basidiospores [25,2,1] 7.5–9.0 × 4.0–4.2 µm, avl × avw = 8.3 × 4.0 µm, Q = 1.7–2.2, avQ = 2.0, oblong to cylindrical, with rounded apex, with distinct spur at base, with or without lateral hilar appendage, in frontal view oblong, hyaline, slightly thick – walled, dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl blue. Basidia 15–18 × 6.0–8.0 µm, clavate, 4-spored. Lamella edge sterile. Cheilocystidia 16–25 × 5–7 µm, clavate to narrowly clavate, often utriform, colorless. Pleurocystidia absent. Pileus covering a trichoderm made up of cylindrical to narrowly clavate elements, 60–150 × 6–15 µm, slightly thick-walled, with pale brown parietal pigment, with under layer made up of hyaline to pale brown-walled, 3–6 µm wide, hyphae. Stipe covering a trichoderm, similar to pileus covering. Clamp connections present in all tissues.

Habitat and distribution—growing in a small group, saprotrophic and terrestrial on nutrient-rich soil, in deciduous rain forest with dominant *Lithocarpus* spp. In Thailand known from one locality. Found in Papua New Guinea (Horak 1981), and reported from India (Natarajan & Manjula 1983). This is a new record for Thailand.

Material examined: **THAILAND**, **Chiang Mai Prov.**, Mae Taeng Distr., Pha Deng Village, 19°07′13.7″N, 98°43′52.9″E, 905 m alt., 11.VII.2007, P. Sysouphanthong, MFLU090014.

Discussion—*Lepiota infelix*, originally described from Papua New Guinea by Horak (1981), is characterized by reddish brown pileus and stipe squamules. The species resembles *L. ignipes* in pileus color, but the slender basidiospore shape in *L. infelix* separates the two taxa.

The also similar *L. alopochroa*, described from Sri Lanka and recorded from New Zealand (as *Lepiotula alopochroa*, Horak 1980), Papua New Guinea (Horak 1981), and now northern Thailand, differs in the color of the pileus squamules.

Lepiota papillata Sysouphanthong, K. D. Hyde, Chukeatirote & Vellinga, **sp. nov.**

Mycobank MB 519794 Fig. 10

Pileus 9–15 mm, conico-convexus dein campanulatus, umbonatus, vel papillatus, squamuloso-fibrillosus, brunneo-griseus vel olivaceo-brunneus. Lamellae liberae, albae dein pallide luteae. Stipes 25–30 × 1.5–2 mm, cylindricus vel subclavatus, squamuloso-fibrillosus. Basidiosporae 7.2–11.8 × 4.0–4.8 µm, calcaratae. Cheilocystidia 30–52 × 6–29 µm, cylindracea, clavata. Epicutis ex cellulis substrictis fusoideo-cylindraceis. Fibulae adsunt.

Holotypus: Thailand, Chiang Mai Prov., Mae Taeng Distr., Hot Spring National Park, 16°06′16.1″N, 99°43′07.9″E, 780–805 m alt., 9.VIII.2007, P. Sysouphanthong PNG041 (MFLU090041, **holotype**).

Etymology: '*papillata*' means with a papilla, referring to the shape of the pileus and of the pileus covering elements.

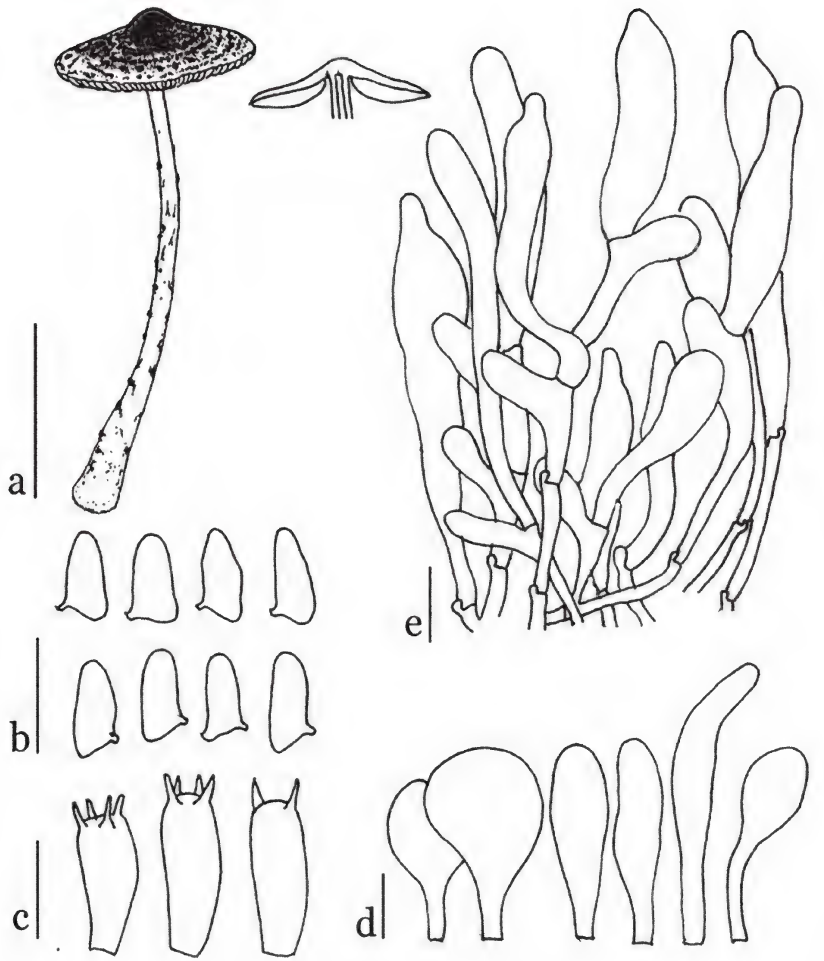

Figure 10. *Lepiota papillata* (MFLU090041).
a. basidioma; b. basidiospores; c. basidia; d. cheilocystidia; e. pileus covering.
Scale bars: a = 10 mm; b–e = 10 μm.

Pileus 9–15 mm, conical at first, expanding to campanulate, umbonate with umbo or high papilla, with inflexed margin, applanate to slightly plano-concave with uplifted margin; background brown (5F4), white to yellowish brown (5E5) on margin, covered with crowded brownish gray to olive brown (4F2–3) squamules at umbo, with brown (6E6) fibrillose squamules around umbo toward margin; margin split, slightly appendiculate, exceeding lamellae. Lamellae free, slightly crowded, ventricose,1.5–2 mm wide, when young white, yellowish

white to pale yellow (4A2–3) when mature, turning grayish orange (5B5) when dried, with serrate concolorous edge. Stipe 25–30 × 1.5–2 mm, cylindrical or slightly tapering upward, with orange-white to grayish red (5A2–7B4) background at apex, grayish orange to brownish orange (5B4–6C5) at center to base, turning brown (6D7) when bruised, covered with brownish gray to olive-brown (4F2–3) squamules from annular zone to base, with orange-white to brownish orange (5A2–6C5) fibrils between squamules; hollow. Annulus with annular zone, with squamules as stipe covering. Context in pileus white, less than 1 mm wide; in stipe grayish orange (5–6B6). Smell grass-like. Taste mild. Spore print white.

Basidiospores [50,2,1] 7.2–11.8 × 4.0–4.8 µm, avl × avw = 8.2 × 4.1 µm, Q = 1.8–2.5, avQ= 2.0, with straight or outgrown spur, cylindrical to oblong, with rounded or more acute apex, in frontal view oblong to cylindrical fusiform, with or without hilar appendage, hyaline, slightly thick-walled, not strongly dextrinoid in Melzer's reagent, congophilous, cyanophilous, not metachromatic in Cresyl blue. Basidia 12–20 × 5.5–7.5 µm, clavate, 4-spored, rarely 2–spored. Lamella edge sterile. Cheilocystidia 30–52 × 6–29 µm, variable in shape, clavate to narrowly clavate, slightly cylindrical, sometimes pyriform, spathulate, rarely narrowly clavate with long pedicel, hyaline, thin – walled. Pleurocystidia absent. Pileus covering a trichoderm made up of erect narrowly clavate elements 30–130 × 5.5–23 µm, with rounded apex, often with abrupt apical excrescence, pale brown-walled, with parietal and intracellular brown pigment, with cylindrical hyphae in under layer, thin-walled, 2.5–5.0 µm wide. Stipe covering in squamules a trichoderm similar to pileus covering. Clamp connections present in all tissues.

Habitat and distribution—growing in small group, saprotrophic and terrestrial, on humus-rich, nutrient-rich soil and dead leaves, found during rainy season in rain forest with dominant bamboo. Only known from one locality in northern Thailand.

Discussion—*Lepiota papillata* resembles *L. griseovirens* but differs especially in the pyriform, spathuliform to narrowly clavate shape of the cheilocystidia (which are narrowly utriform, obovoid, to nearly cylindrical cheilocystidia in *L. griseovirens;* Vellinga 2001) and in the abrupt apical excrescences often found on the pileus covering elements.

Lepiota grangei (Eyre) Kühner comes close in morphology but differs in the dark gray-green, grayish blue, green-brown, or blue-brown to brown pileus, the cylindrical to utriform cheilocystidia, and the intracellular pigment in the long pileus covering elements.

This species comes very close to *L. tomentella* J.E. Lange in pileus color and basidiospore size, but the latter has longer pileus covering elements that lack abrupt apical excrescences (Vellinga & Huijser 1993).

Lepiota pilodes Vellinga & Huijser differs from *L. papillata* by dull orange-brown to dark brown and ochraceous brown colors at centre of pileus, and longer pileus- and stipe-covering elements (60–280(–330) × (6.5–)8.0–21 µm; Vellinga & Huijser 1993, Vellinga 2001).

Lepiota poliochloodes Vellinga & Huijser, Persoonia 15: 229. 1993. FIG. 11

MISAPPLIED NAMES: *Lepiota griseovirens* sensu Bon (1993), Reid (1972); *Lepiota griseovirens* var. *griseovirens* sensu Bon (1981).

PILEUS 12–25 mm, campanulate to umbonate, applanate to plano-concave with wide umbo, with straight margin, at centre with crowded squamules to small plush-like squamules or floccules, grayish brown, grayish green brown to dark brown (Mu. 2.5 Y 4–5/2, 6E3–6, 6F5–6), with squamules or fibrils around umbo toward margin and distant at marginal zone, on brownish orange, slightly orange or light brown fibrillose background (5C4, 6D4); margin peeling from background when mature, exceeding lamellae. LAMELLAE, L = around 40, l = 0–3, crowded to moderately crowded, just free, ventricose, 1.7–3 mm wide, white to yellowish white, distinctly pinkish cream (4A2, Mu. 10 YR 8/6), with eroded to floccose, distinctly cystidiose edge. STIPE 25–36 × 1–2.5 mm, cylindrical and slightly wider at middle, with white or yellowish white to orange-white or pale pink (4A2, 5A2) fibrillose background, with dark brown to dark gray-green (6F5–6) squamules from annular zone to base, with white to yellowish white (4A2) fibrils between squamules, hollow, with white rhizomorphs at base. ANNULUS an annular zone, with gray-green to dark brown squamules. CONTEXT in pileus white to pale cream, 1.5–2 mm wide, in stipe white, yellowish white (4A2) to pale orange. SMELL mild. TASTE unknown. SPORE PRINT WHITE.

BASIDIOSPORES [70,3,3] 6.2–8.2 × 3.0–3.8 µm, avl × avw = 7.2 × 3.5 µm, Q = 1.6–2.4, Qav = 2.0, in side-view cylindrical or narrowly triangular with or without lateral spur, but spur not protruding abaxially, and with rather abrupt base, in frontal view cylindrical, with rounded apex or tapering toward apex, with 1 or 2 guttules, congophilous, dextrinoid, cyanophilous, not metachromatic in Cresyl Blue. BASIDIA 15–19 × 6 – 8 µm, 4-spored, rarely 2-spored, relatively short and plump. LAMELLA EDGE sterile. CHEILOCYSTIDIA 18–40 × 3–9 µm, mostly cylindrical, to very narrowly clavate, some narrowly lageniform or slightly fusiform, strangulate, digitate. Pleurocystidia absent. PILEUS AND STIPE COVERING a trichoderm or slightly irregular trichoderm made up of erect cylindrical, narrowly clavate to narrowly lageniform elements, (20–)30–130 × 6.5–15 µm, with pale brown walls, and with intracellular brown pigment, thin-walled, with under layer made up of cylindrical, hyaline, 2.5–9.0 µm wide hyphae. CLAMP CONNECTIONS present in all tissues.

HABITAT AND DISTRIBUTION—growing solitary, saprotrophic and terrestrial in deciduous forest and bamboo forest. Reported from the Netherlands,

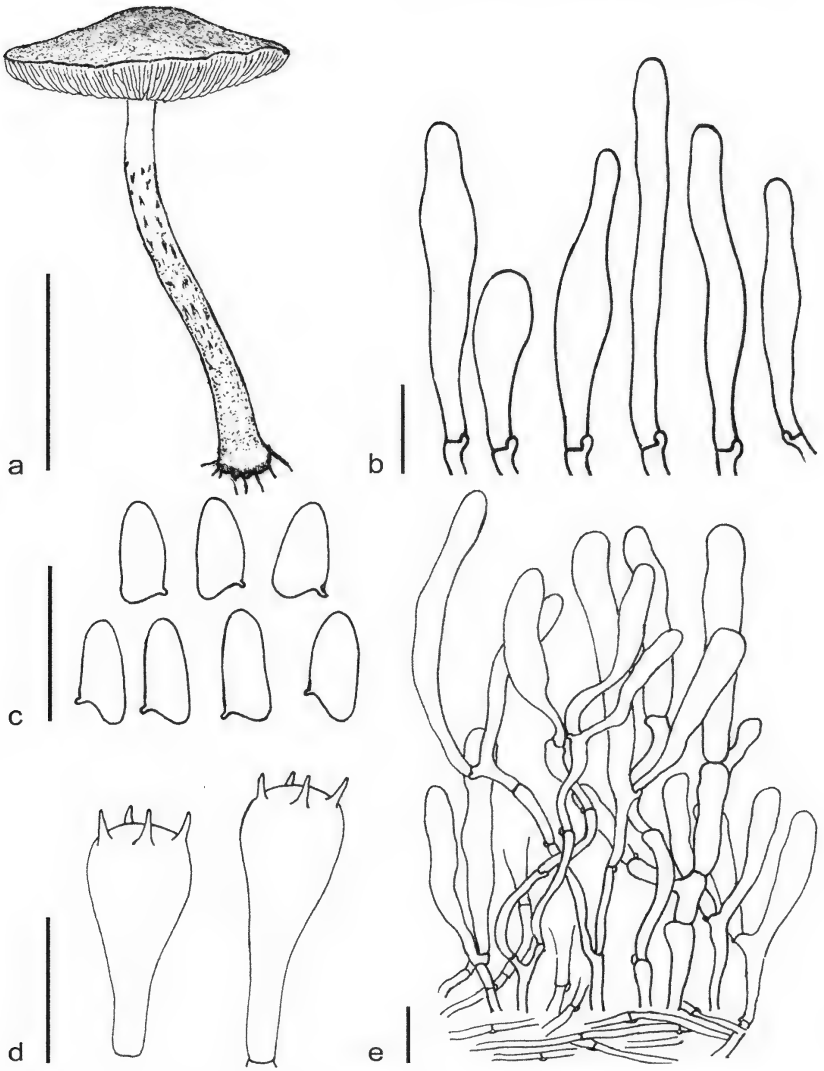

Figure 11. *Lepiota poliochloodes* (MFLU090182).
a. basidioma; b. cheilocystidia; c. basidiospores; d. basidia; e. pileus covering.
Scale bars: a = 20 mm; b–d = 10 µm; e = 20 µm.

Denmark, France, and Great Britain (Vellinga 2001); this is the first record for Thailand.

Material examined: **THAILAND, Chiang Mai Prov.**, Mae Taeng Distr., Mae Sae village, 19°07′13.7″N, 98°43′52.9″E, 905 m alt., 8.VIII.2008, P. Sysouphanthong,

MFLU090182; Mok Fah Waterfall National Park, 20°02′43.1″N, 99°52′35.0″E, 596 m alt., 5.VII.2008, P. Sysouphanthong, MFLU090118; Mae Rim Distr., Mae Sa Valley, 12.VII.2008, J-K. Liu (collection ecv3877), MFLU081272.

Discussion—*Lepiota poliochloodes* is rare in Northern Thailand, where it is known from three localities. The macro- and micromorphology of the Thai material agree with the description of *L. poliochloodes* from Europe (Vellinga & Huijser 1993); unfortunately, there are no European nrITS sequence data available for comparison.

The relatively small spores and short pileus-covering elements combine with the pileus with grayish brown to green colors over a slightly orange background to characterize this species.

Lepiota griseovirens, which has similarly sized basidiospores and cheilocystidia differs from *L. poliochloodes* in the much darker basidiomata and the longer pileipellis elements.

***Lepiota* sp. 1** Fig. 12

Pileus 15 mm, plano-convex with umbo and slightly inflexed margin, at umbo not completely closed, with tufted scales; around umbo scales are more spreading out on yellowish background; scales small and reddish brown (Mu. 2.5 YR 3/4–6). Lamellae, L = around 35, l = 1–3, free, not very crowded, slightly ventricose, yellowish (Mu. 10 YR–2.5 Y 8/4) with lighter, slightly cystidiose edge. Stipe 24 × 2 mm, cylindrical, above annular zone innately lengthwise fibrillose, pale brown, below annular zone with small patches, sometimes spiny, of same material and color as on pileus, hollow, with white rhizomorphs. Smell a bit unpleasant, when old astringent.

Basidiospores [20,1,1] in side view 7.1–8.4 × 2.8–4.2 µm, avl × avw = 7.8 × 3.2 µm, Q = 1.9–2.8, avQ = 2.4, with distinct basal spur, in some spores spur also abaxially bulging, with round to slightly amygdaliform apex, in frontal view obovoid to subcylindrical, thick-walled, smooth, dextrinoid, and congophilous. Basidia 15–22 × 6.5–8.0 µm, 4-spored, some 2-spored, with basal clamp connection. Lamella edge sterile. Cheilocystidia 22–28 × 7.0–10.5 µm, utriform, fusi-utriform, and narrowly clavate, with basal clamp-connection. Pleurocystidia absent. Pileus covering trichodermal, made up of erect elements, 52–170 × 7.5–12.5 µm, cylindrical with rounded apex, almost always narrowed into pedicel, some very narrowly lageniform, with intracellular brown pigment. Stipe covering similar to pileus covering. Clamp connections present in all tissues.

Habitat and distribution—solitary, saprotrophic on humus rich soil in orchard with bamboo; only collected once close to Chiang Mai.

Material examined: **THAILAND, Chiang Mai Prov.**, Mae Rim Distr., Mae Sa Valley, 12.VII.2008, leg. J-K. Liu (collection E.C. Vellinga 3881), MFLU08-1259.

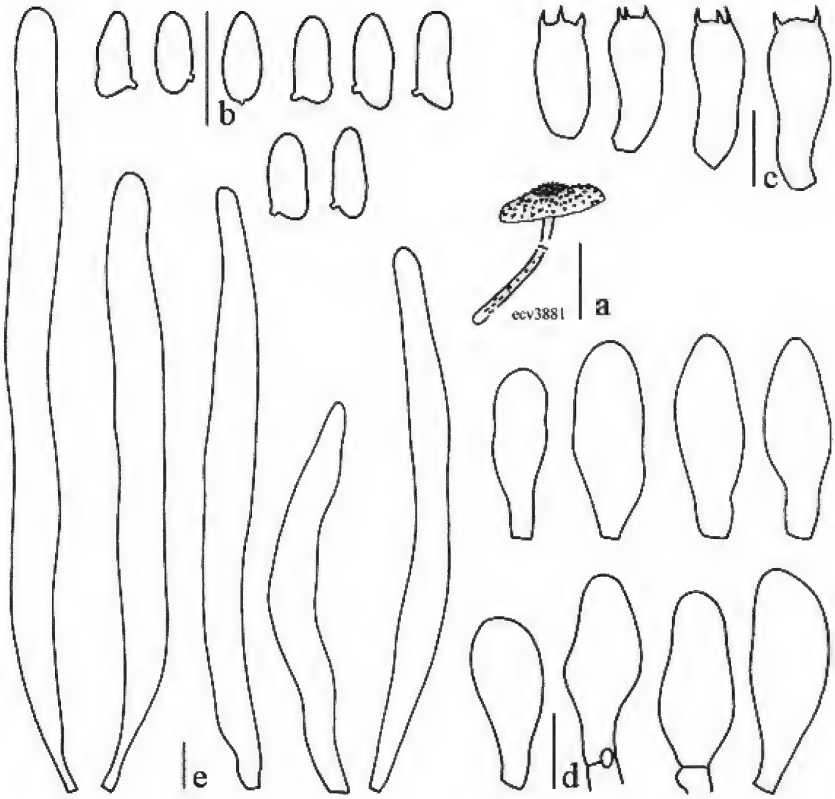

Figure 12. *Lepiota* sp. 1 (MFLU081259).
a. basidioma; b. cheilocystidia; c. basidiospores; d. basidia; e. pileus covering elements.
Scale bars: a = 1 cm; b–d = 10 μm.

Discussion—Macroscopically, this undescribed taxon resembles *L. echinella* Quél. & G.E. Bernard because of the tufted reddish brown pileus scales but differs in the spurred spores and shorter elements in the pileus covering.

We refrain from describing this taxon as a new species, as the sole collection contained only one specimen.

***Lepiota* sp. 2** Fig. 13

Pileus 30 mm, campanulate or umbonate with broad umbo, with straight margin, covered with crowded grayish brown to slightly reddish brown (8F6–8) squamules at center and around umbo toward margin and there more radially fibrillose, on grayish yellow (4B3), white to yellowish white (4A2), radially

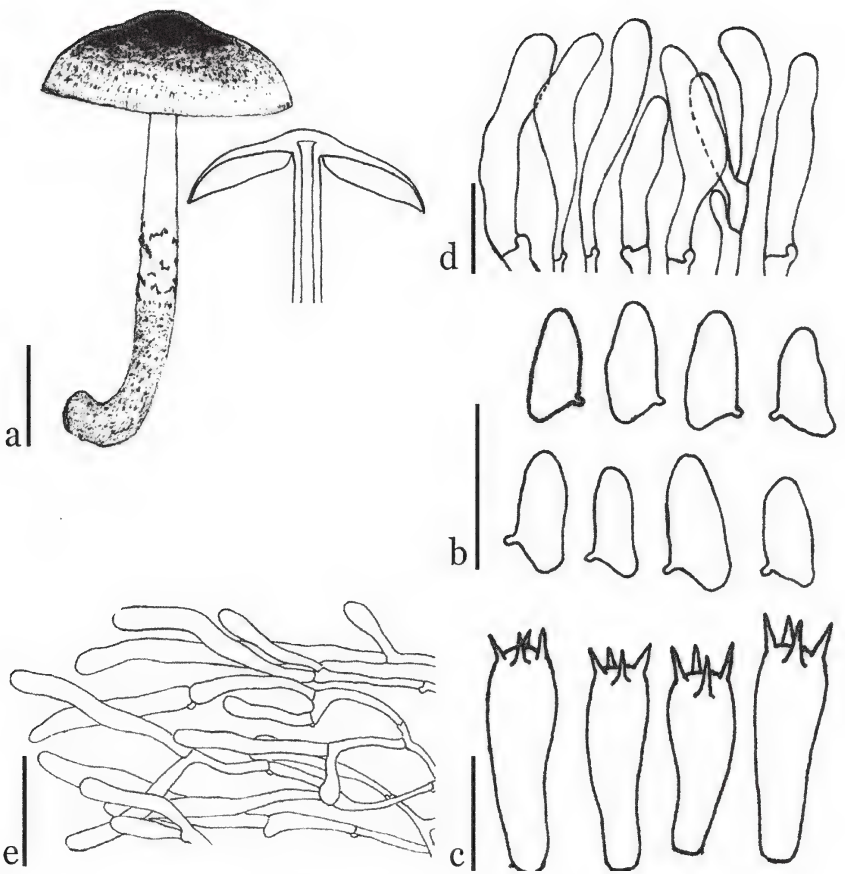

Figure 13. *Lepiota* sp. 2 (MFLU090006).
a. basidioma and context; b. cheilocystidia; c. basidiospores; d. basidia; e. pileus covering.
Scale bars: a = 10 mm; b–d = 10 μm; e = 20 μm.

fibrillose background; margin exceeding lamellae. Lamellae free, crowded, broadly ventricose, 2.5–3 mm wide, white to yellowish white (3A2), with slightly wavy edge. Stipe 40 × 3–4 mm, cylindrical, slightly wider at center and tapering to apex and curved at base; surface with white to pale orange (5A3) fibrils, with squamules from middle downwards, concolorous with squamules on pileus, with yellowish brown (5E5) fibrillose squamules at basal zone. Context in pileus thick at umbo, 4 mm wide, white to yellowish white (3A2); in stipe pale orange (6A3), hollow. Smell *Termitomyces*-like. Taste sweet. Spore print white.

Basidiospores [25,1,1] 6.2–8.0 × 3.2–4.0 µm, avl × avw = 7.3 × 3.7 µm, Q = 1.6–2.5, Qav = 1.9, in side-view with truncate to spurred base, oblong to cylindrical triangular or with curved abaxial side, sometimes with rounded or acute apex, in frontal view oval or cylindrical, strongly dextrinoid, congophilous, cyanophilous, not metachromatic in Cresyl blue. Basidia 16–21 × 7.0–8.0 µm, narrowly clavate, 4–spored, hyaline, thin-walled. Lamella edge sterile, with crowded cheilocystidia. Cheilocystidia 12–45 × 5.2–10 µm, cylindrical, sometimes narrowly lageniform, narrowly clavate, with rounded apex, subcapitate, sometimes slightly swollen at center, utriform, colorless, thin-walled. Pleurocystidia absent. Pileus covering a cutis made up of irregular narrowly clavate hyphae, 25–128 × 10–18 µm, usually narrowed into pedicel, with rounded or subcapitate apex, slightly thick-walled, with or without septum, with basal clamp connections, with yellowish brown parietal pigment; under layer made up of cylindrical 3.0–7.0 µm wide hyphae, thin-walled, with yellowish brown walls in upper part, with hyaline walls in lower part. Stipe covering a cutis similar to pileus covering. Clamp connections present in all tissues.

Habitat and distribution—growing solitary, saprotrophic and terrestrial on humus-rich soil with dead leaves and wood, in deciduous forest with *Lithocarpus* spp. Only found in one locality in northern Thailand.

Material examined: **THAILAND**, **Chiang Mai Prov.**, Mae Taeng Distr., Pha Deng Village, 19°07′13.7″N, 98°43′52.9″E, 905 m alt., 19.VII.2007, P. Sysouphanthong, MFLU090006.

Discussion—This undescribed taxon is characterized by grayish brown to reddish brown squamules on pileus and stipe, truncate to spurred basidiospores, cylindrical to narrowly clavate cheilocystidia, and a cutis-like pileus covering.

Lepiota griseovirens, which can be confused with this species in the field because of the color of the pileus squamules, differs in the trichodermal pileus covering.

Here also the single collection comprised only one basidioma, and we were unable to obtain nrITS sequence data. We await further collections before proposing a name.

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We wish to acknowledge the Mushroom Research Centre in Mae Taeng District, Chiang Mai Province, Thailand for providing funding, and facilities for collecting and study. The International Fungal Research and Development Centre, The Research Institute of Insect Resources (RIRI) is thanked for providing materials and laboratory facility for the molecular work. Funding of ECV and PS in 2007–2009 by NSF grant DEB 0618293 is gratefully acknowledged. This study was also partially supported by the project "Value added products from Basidiomycetes: Putting Thailand's biodiversity to use" (BRN049/2553). The comments by the two reviewers, Dr R.-L. Zhao and Dr Zai-Wei Ge, are gratefully acknowledged.

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New records of *Digitoramispora* from China

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Abstract — Four species collected from plant debris in natural areas of southern China are recorded for the first time from China. *Digitoramispora caribensis*, *D. tambdisurlensis*, *D. excentrica*, and *D. lageniformis* are described and illustrated, and a key to currently accepted *Digitoramispora* species is provided. The specimens are deposited in Herbarium of Shandong Agricultural University, Plant Pathology (HSAUP), and Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS).

Key words — anamorphic fungi, taxonomy

Introduction

Castañeda & Kendrick (1990) introduced *Digitoramispora* to accommodate two species, *D. caribensis* (type species) and *D. excentrica*. Two other species, *D. lageniformis* and *D. tambdisurlensis* were described from Thailand and India, respectively (Somrithipol & Jones 2003, Pratibha et al. 2009). Until now, *Digitoramispora* has contained only these four species with no reports from China. *Digitoramispora* species produce dictyosporous, digitate or irregular conidia with peripheral hyaline or colourless short radiating branches or cells, and irregularly (often percurrently) extending conidiophores (Castañeda & Kendrick 1990). During investigations of tropical fungi from the forests of southern China, all four species of *Digitoramispora* were collected. They are illustrated and described as new records for China.

Taxonomy

Digitoramispora caribensis R. F. Castañeda & W.B. Kendr., University of Waterloo Biology Series 33: 20 (1990) Fig. 1

Colonies on the natural substrate effuse, black. Mycelium partly superficial, partly immersed in the substrate, composed of smooth, brown, branched, septate hyphae, 1–2 µm wide. Conidiophores macronematous, mononematous,

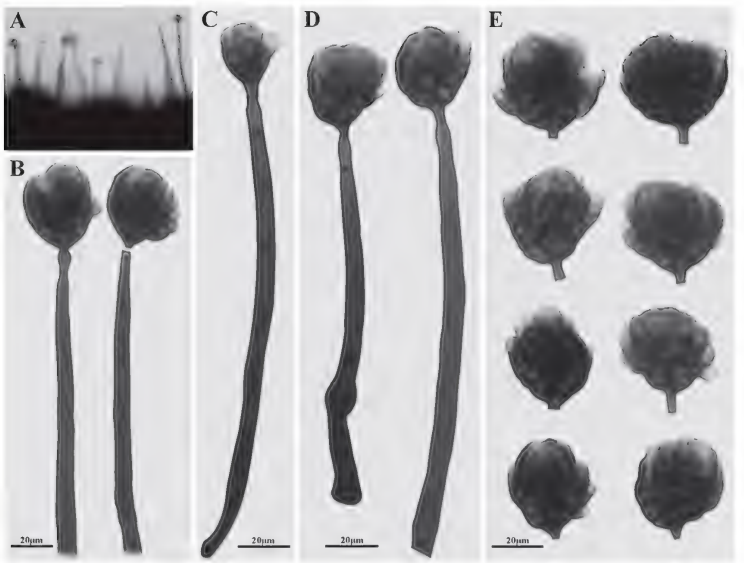

Fig. 1. *Digitoramispora caribensis* A. Colonies on the natural substratum. B–D. Conidiophores, conidiogenous cells and conidia. E. Mature conidia.

single, straight to slightly flexuous, unbranched, dark brown, often swollen at the base, up to 110 μm long, 3–5 μm thick. Conidiogenous cells monoblastic, integrated, terminal, cylindrical to lageniform, 6–12 × 2–2.5 μm. Conidia holoblastic, digitate, lobed at the apex, 17–21 × 14–20 μm, dark brown at the base and centre, pale brown to colourless at apical cells and short branches, seceding schizolytically.

Specimen examined: CHINA. Gungdong Province: Mountain Danxia, on dead branches of unidentified plant, 22 Oct. 2010, L.Y. Sun, HSAUP H3273 (duplicate HMAS 146118).

Comments—*Digitoramispora caribensis,* the type species of *Digitoramispora,* was previously known only from Cuba. Our specimen is similar to the type species, but it lacks annellidic conidiogenous cells and its conidia are somewhat larger (17–21 × 14–20 μm vs. 15–20 × 10–15 μm).

Digitoramispora tambdisurlensis Pratibha, Raghuk. & Bhat, Mycotaxon 107: 383 (2009) Fig. 2

Colonies on the natural substrate effuse, dark brown. Mycelium immersed in the substrate, composed of smooth, brown, branched, septate hyphae, 2–3 μm wide. Conidiophores macronematous, mononematous, straight to slightly flexuous, unbranched, dark brown, swollen at the base, 200–230 × 5.5–11 μm.

Fig. 2. *Digitoramispora tambdisurlensis* A. Colonies on the natural substratum. B. Conidiophores and conidiogenous cells. C. Conidiophores with conidia. D. Conidia

Conidiogenous cells monoblastic, integrated, terminal, cylindrical, producing only one terminal conidium, 9–12.5 × 4.5–6 μm. Conidia holoblastic, muriform, digitate, variable in shape, dark brown at the base and centre with peripheral light brown cells or branches, 64.5–80 × 38.5–55.5 μm.

Specimen examined: CHINA. Hunan Province: forest park of Zhang jiajie, on dead branches of unidentified plant, 18 Aug. 2010, Y.D. Zhang, HSAUP H3168 (duplicate HMAS 146119).

Comments—*Digitoramispora tambdisurlensis* differs from the other three species in the genus by its larger conidia and conidiophores. The shape and size of the conidia in our collection compare well with those described by Pratibha et al. (2009). The only difference is that the conidia and conidiophores in our collection are slightly smaller.

Digitoramispora excentrica (B. Sutton) R.F. Castañeda & W.B. Kendr., University of Waterloo Biology Series 33: 20 (1990) Fig. 3

Colonies on natural substrate effuse, black. Mycelium partly superficial, partly immersed in the substrate, composed of smooth, brown, branched, septate hyphae, 1–2 μm wide. Conidiophores macronematous, mononematous, single, straight to slightly flexuous, unbranched, dark brown, often swollen at the base, up to 140 μm long, 3–4 μm thick, with up to 5 lageniform or doliiform

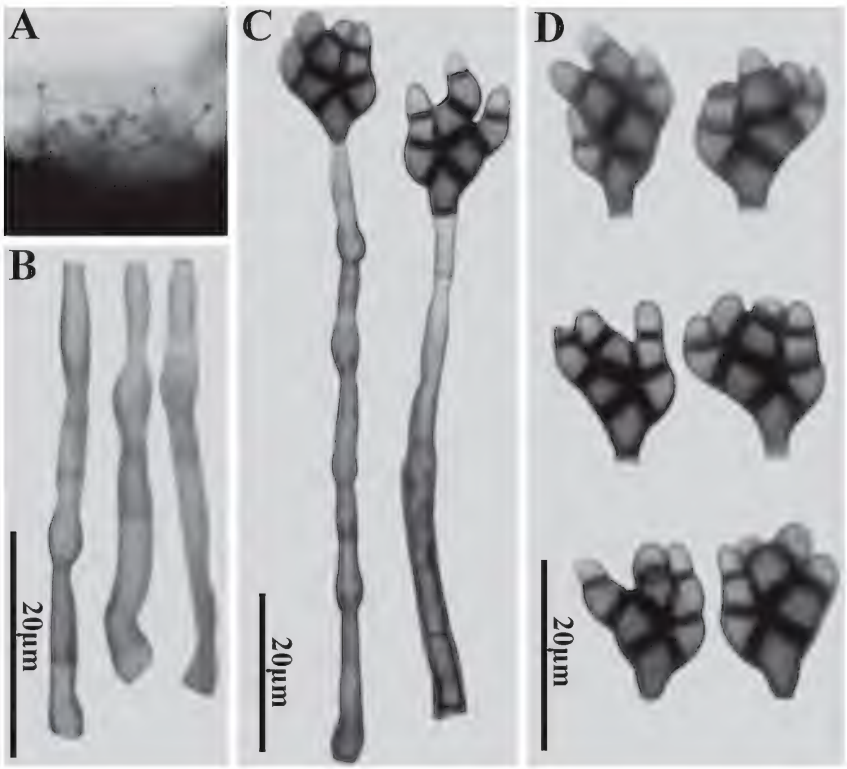

FIG. 3. *Digitoramispora excentrica* A. Colonies on the natural substratum. B. Conidiophores and conidiogenous cells. C. Conidiophores with conidia. D. Conidia.

percurrent proliferations. Conidiogenous cells monoblastic, integrated, terminal, cylindrical to lageniform, 7–10 × 2–2.5 μm. Conidia holoblastic, muriform or cheiroid, lobed at the apex, variable in shape, with 1–2 transverse septa and 1–3 longitudinal septa, the septa are partly obscured by a band of wall which is more deeply pigmented and almost black, apical cell of each branch colourless, 16.5–21 × 11.5–14 μm.

SPECIMEN EXAMINED: CHINA. GUNGDONG PROVINCE: Mountain Danxia, on dead branches of unidentified plant, 22 Oct. 2010, Y.D. Zhang, HSAUP H3273 (duplicate HMAS 146120).

COMMENTS—*Digitoramispora excentrica* was originally described as *Acrodictys excentrica* by Sutton (1969) in Canada. Hughes (1979) transferred it to *Arachnophora* (as *A. excentrica* (B. Sutton) S. Hughes) based on the rhexolytic conidial secession. Castañeda & Kendrick (1990) provided the combination *Digitoramispora excentrica* based on the percurrently proliferating

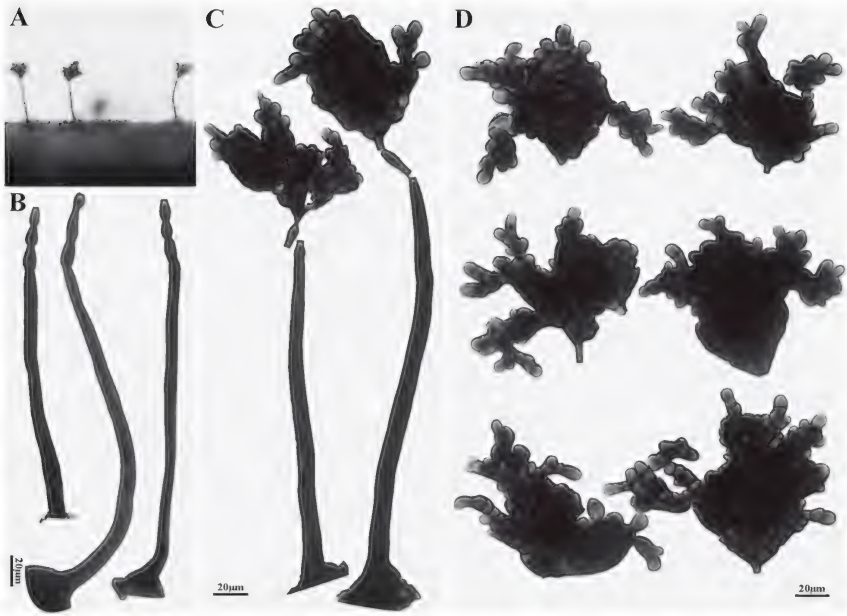

FIG. 4. *Digitoramispora lageniformis* A. Colonies on the natural substratum. B. Conidiophores and conidiogenous cells. C. Conidiophores with conidia. D. Conidia.

conidiophores and dictyosporous, digitate conidia with colourless apical cells. Our specimen lacks the *Selenosporella*-like synanamorph at the apex. The conidial shape is similar to the type specimen, but the conidia are somewhat longer (16.5–21 μm vs. 13–18 μm) than those of the type.

Digitoramispora lageniformis Somrith. & E.B.G. Jones, Nova Hedwigia 77: 374 (2003) FIG. 4

Colonies on natural substrate effuse, black. Mycelium partly superficial, partly immersed in the substrate, composed of smooth, brown, branched, septate hyphae, 1.5–3 μm wide. Conidiophores macronematous, mononematous, straight to slightly flexuous, unbranched, dark brown, often swollen at the base, up to 260 μm long, 6–10.5 μm thick, with up to 3 lageniform or doliiform percurrent proliferations. Conidiogenous cells monoblastic, integrated, terminal, producing only one terminal conidium, cylindrical to lageniform, 12–17 × 4–6 μm. Conidia holoblastic, muriform or digitate, variable in shape, 38–57 × 40–51 μm, with many short, 1–3 septate, aggregated branches, brown or dark brown at the base and in the centre, apical cell of each branch colourless.

SPECIMEN EXAMINED: CHINA. GUNGDONG PROVINCE: Mountain Danxia, on dead branches of unidentified plant, 22 Oct. 2010, L.Y. Sun, HSAUP H7034 (duplicate HMAS 146121).

Comments—*Digitoramispora lageniformis* is distinguished from other species by the lageniform or doliiform percurrently proliferating conidiophores and numerous short branches on the conidia. Compared with the morphology of the type specimen described by Somrithipol & Jones (2003), the conidia in our collection are slightly larger.

Key to *Digitoramispora* species

1. Conidiogenous cells without proliferation 2
   Conidiogenous cells with lageniform or doliiform proliferations 3
2. Conidia 15–20 µm long, 10–15 µm wide .....*D. caribensis*
   Conidia 50–90 µm long, 40–75 µm wide .....*D. tambdisurlensis*
3. Conidia 13–18 µm long, 12–17 µm wide ..... *D. excentrica*
   Conidia 37–45 µm long, 28–32 µm wide ..... *D. lageniformis*

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Seven new records of foliicolous lichens from Vietnam

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Abstract — Seven foliicolous species growing in tropical regions of Vietnam are reported as new to the country. Described are *Arthonia accolens, Calenia aspidota, Calopadia subcoerulescens, Coenogonium minimum, Fellhanera rhapidophylli, F. semecarpi,* and *Porina subnitidula.* Among them, *Coenogonium minimum* and *Porina subnitidula* are reported for the first time from the paleotropics.

Key words — *Ascomycota,* geographical distribution, lichen-forming fungi, taxonomy

Introduction

The present paper, which is a further report on the taxonomic study of foliicolous lichens from Vietnam by Nguyen et al. (2010, 2011), describes seven species newly recorded from three different localities [Thác Đray Sáp (= Dray Sap waterfall), Vườn quốc gia Bạch Mã (= Bach Ma National Park), and Vườn quốc gia Phong Nha-Kẻ Bàng (= Phong Nha Ke Bang National Park)] in Vietnam. The discovery in Vietnam of *Coenogonium minimum* and *Porina subnitidula,* previously known only from the Neotropics, provides further information about their distribution in southeastern Asia. In addition to the newly recorded species, the authors also collected previously reported (by Santesson 1952, Vězda 1977, Farkas & Sipman 1993, 1997, Lücking & Vězda 1998, Aptroot & Sparrius 2006) the foliicolous lichens *Bacidina apiahica* (Müll. Arg.) Vězda, *B. pallidocarnea* (Müll. Arg.) Vězda, *Byssoloma chlorinum* (Vain.)

Zahlbr., *Calopadia puiggarii* (Müll. Arg.) Vězda, *Coenogonium dilucidum* (Kremp.) Kalb & Lücking, *C. subluteum* (Rehm) Kalb & Lücking, *Echinoplaca epiphylla* Fée, *Porina corruscans* (Rehm) R. Sant., *P. diaphana* Vězda, *P. nitidula* Müll. Arg., *Sporopodium phyllocharis* (Mont.) A. Massal., *Strigula orbicularis* Fr., *S. nitidula* Mont., and *Trichothelium alboatrum* Vain. The most common and widely distributed species found during the survey were *Bacidina apiahica, Calopadia puiggarii, Coenogonium dilucidum, Porina nitidula,* and *Strigula nitidula.*

This is the first report of lichens from central Vietnam. Earlier workers (Santesson 1952, Vězda 1977, Farkas & Sipman 1993, 1997, Lücking & Vězda 1998, Aptroot & Sparrius 2006) have reported Vietnamese lichens mostly from the northern parts of the country. Surveys of the several provinces still left unexplored will definitely contribute further to our knowledge of the Vietnamese lichen flora. Brief taxonomic descriptions, chemical and ecological notes, and comments are provided below for each of the newly recorded species.

Materials & methods

The lichen samples were collected from three different localities between 22 July 2006 and 08 February 2010. The study area covers the three Vietnamese provinces Daknong, Quang Binh, and Thua Thien Hue. After drying at room temperature, the lichen samples were identified using stereo and light microscopes: a NIKON Eclipse E200 dissecting microscope was used for identifying morphological characters of thallus, reproductive structures, color, size and shapes, while an OLYMPUS BX 50 compound microscope was used for studying the anatomy of thalli and fruiting bodies. All measurements were made from material mounted in water and stained in lactophenol cotton blue (LCB). An average of ten measurements per structure was recorded for the sizes of thallus, ascomata, and ascospores and the thicknesses of hymenium, hypothecium, exciple, and involucrellum. Only free ascospores lying outside the asci were measured. Ascospore dimensions are generally presented as minimum value observed – maximum value observed. Spot test reactions were carried out on hand sections of thalli and apothecia under the compound microscope. Iodine (I) was used to check the color reactions of the ascus wall and the hymenium. Thin Layer Chromatography (TLC) was performed in solvent system C (Toluene:Acetic acid 85:15) as described by Orange et al. (2010). Terminology used in this study follows Lücking (2008). Vouchers have been deposited in the herbarium of the Lichen & Allied Bioresource Center at the Korean Lichen Research Institute (KoLRI), Sunchon National University, South Korea.

New records

Arthonia accolens Stirt., Proc. Roy. Phil. Soc. Glasgow 11: 105, 1879

Thallus dispersed into rounded patches, smooth, 2–20 mm across, ecorticate, brownish green. Photobiont cells rectangular in radiate plates. Apothecia adnate, rounded, 0.1–0.3 mm diam., dark brown. Hypothecium 2–4 μm high,

brown. Hymenium pale brown. Asci globose, 15–20 × 13–15 µm, 8-spored. Ascospores obovate, hyaline, 2-septate, 10–12 × 3–4 µm, distal cell enlarged.

Chemistry — Spot test reactions: thallus and apothecia K–, C–, KC–, P–, N–. Epithecium N–. Secondary metabolites: none detected.

Distribution & ecology — Pantropical (Lücking 2008); new to Vietnam. At the collection site the species was found growing on the understory leaves in lowland tropical rain forest along with *Coenogonium dilucidum* and *Porina* spp.

Specimen Examined – **VIETNAM: Thua Thien Hue Province**: Vườn quốc gia Bạch Mã, 16°12′55.50″N, 107°51′40.20″E, alt. 685 m, on leaf of *Ochna* spp., 08 February 2010, T. Nguyen 100001 (KoLRI).

Remarks — *Arthonia cyanea* Müll. Arg., another *Arthonia* species known from Vietnam, differs in having bluish to greenish gray, white pruinose apothecia. The apothecia in *A. accolens* are light to dark brown colored and never pruinose. The species can also be confused with *A. leptosperma* (Müll. Arg.) R. Sant., which differs in having smaller, 1-septate ascospores and a continuous thallus with net-like photobiont. For further descriptions of *A. accolens,* see Lücking (2008).

Calenia aspidota (Vain.) Vězda, Folia geobot. phytotax. 19: 195, 1984

Thallus dispersed into rounded patches, 5–13 mm across, greenish gray, 47–80 µm thick, cortex formed by rounded cells, strongly inflated with calcium oxalate crystals, white. Setae not observed. Apothecia immersed, zeorine, rounded, 0.2–0.5 mm diam. and 70–120 µm high; disc whitish gray with white pruina; margin distinct, irregularly lobulate, white. Hymenium 90–100 µm, hyaline. Epithecium 3–5 µm thick, with algal layer. Asci ellipsoid, 70–85 × 20–35 µm, single spored. Ascospores hyaline, muriform, 50–80 × 15–30 µm. Hyphophores white, 0.2–0.4 mm high.

Chemistry — Spot test reactions: thallus and apothecia K–, C–, KC–, P–. Secondary metabolites: none detected.

Distribution & ecology — Pantropical (Lücking 2008); new to Vietnam. At the collection site the species was found growing on the understory leaves in lowland tropical rain forest along with *Calopadia puiggarii* and *Porina nitidula.*

Specimen Examined – **VIETNAM: Daknong Province**: Thác Đray Sáp, 12°32′21.39″N, 107°53′19.80″E, alt. 399 m, on leaf, 22 July 2009, Y. Joshi & T. Nguyen 090183 (KoLRI).

Remarks — *Calenia thelotremella* Vain., another *Calenia* species known from Vietnam, differs in having transversely septate ascospores, a verrucose, pale greenish, or yellowish gray thallus, and epruinose, yellowish gray apothecia. The species can also be confused with *C. bullatinoides* Lücking, which differs in

having 2–4-spored asci and smaller hyphophores with blunt, darkened apices. For further descriptions of *C. aspidota,* see Lücking (2008).

Calopadia subcoerulescens (Zahlbr.) Vězda, Sched. Lichenes Selecti Exsiccati Fascicle 88: 3, no. 2185, 1988

Thallus dispersed into rounded patches, 10–15 mm across, smooth, pale greenish gray. Apothecia rounded, 0.2–0.4 mm diam., plane, brownish black; margin thin, prominent, gray. Excipulum 35–40 µm broad, hyaline, paraplectenchymatous. Hymenium 100–120 µm, hyaline. Hypothecium 30–40 µm high, aeruginous. Apothecial base aeruginous. Epithecium 7–10 µm high. Asci 70–95 × 20–25 µm, single spored. Ascospores hyaline, muriform, 70–85 × 15–20 µm. Campylidia not observed.

Chemistry — Spot test reactions: thallus and apothecia K–, C–, KC–, P–. Secondary metabolites: none detected.

Distribution & ecology — Pantropical (Lücking 2008); new to Vietnam. At the collection site the species was found growing on the understory leaves in lowland tropical rain forest at an elevation of 136 m.

Specimen Examined – **VIETNAM: Quang Binh Province**: Vườn quốc gia Phong Nha-Kẻ Bàng, 17°28′51.60″N, 106°18′39.10″E, alt. 136 m, on leaf of *Lauraceae* tree, 06 February 2010, T. Nguyen 100028b (KoLRI).

Remarks — *Calopadia puiggarii,* another *Calopadia* species known from Vietnam, differs in having a dark brown hypothecium and grayish brown apothecia. *Calopladia subcoerulescens* always has an aeruginous hypothecium and grayish black to black apothecia. For additional details see Lücking (2008).

Coenogonium minimum (Müll. Arg.) Lücking, Fl. Neotrop. Monogr. 103: 572, 2008

Thallus continuous, smooth, green. Apothecia sessile, rounded, 0.1–0.13 mm diam. Disc concave, yellowish brown, slightly translucent; margin distinct, prominent, concolorous with disc. Excipulum 5–8 µm high, pale yellowish brown. Hypothecium 5–8 µm high, hyaline. Hymenium 40–45 µm high, hyaline. Asci 42–48 × 5–6 µm, 8-spored. Ascospores ellipsoid, 1-septate, 12–14 × 3–4 µm. Pycnidia not observed.

Chemistry — Spot test reactions: thallus K–, C–, KC–, P–. Secondary metabolites: none detected.

Distribution & ecology — Neotropical (Lücking 2008); new to Vietnam. At the collection site the species was found growing along with *Coenogonium dilucidum* on the understory leaves in lowland tropical rain forests between elevations of 136–556 m.

Specimens Examined – **VIETNAM: Quang Binh Province**: Vườn quốc gia Phong Nha-Kẻ Bàng, 17°33′07.10″N, 106°18′06.10″E, alt. 556 m, on leaf, 05 February 2010, T. Nguyen 100168h (KoLRI); 17°33′37.10″N, 106°18′06.10″E, alt. 556 m, on leaf of *Rubiaceae*

tree, 06 February 2010, T. Nguyen 100016 (KoLRI); 17°28′51.60″N, 106°18′39.10″E, alt. 136 m, on leaf, 06 February 2010, T. Nguyen 100031, 100066b (KoLRI); 17°33′07.10″N, 106°18′06.10″E, alt. 556 m, on leaf of *Fagaceae* tree, 06 February 2010, T. Nguyen 100081a (KoLRI).

Remarks — *Coenogonium dilucidum, C. disciforme* Papong et al., *C. luteum* (Dicks.) Kalb & Lücking, and *C. subluteum* are the other *Coenogonium* species known from Vietnam. *Coenogonium dilucidum* differs in having wax-colored to pale yellow, slightly concave apothecia; *C. luteum* and *C. subluteum* have larger apothecia (0.2–1.5 mm diam.), while *C. disciforme* differs in bearing numerous disc-shaped isidia. For further descriptions of *C. minimum,* see Lücking (2008).

Fellhanera rhapidophylli (Rehm) Vězda, Folia geobot. phytotax. 21: 214, 1986

Thallus continuous, farinose, greenish gray to green. Apothecia rounded, 12–14 µm diam.; disc plane to slightly convex, grayish brown to dark reddish brown; margin thin, persistent, pale gray. Excipulum paraplectenchymatous, 20–25 µm broad. Hypothecium dark brown. Hymenium colorless, 40–55 µm high. Asci 42–55 × 10–11 µm, 8-spored. Ascospores ellipsoid, 3-septate, 13–16 × 3–4 µm. Pycnidia not observed.

Chemistry — Spot test reactions: thallus K–, C–, KC–, P–. Secondary metabolites: none detected.

Distribution & ecology — Pantropical (Lücking 2008); new to Vietnam. At the collection site *F. rhapidophylli* was found growing on the understory leaves in tropical rain forest along with *F. bouteillei* (Desm.) Vězda.

Specimen Examined – **VIETNAM: Daknong Province**: Thác Đray Sáp, 12°32′21.39″N, 107°53′19.80″E, alt. 685 m, on leaf, 22 July 2009, Y. Joshi & T. Nguyen 090094 (KoLRI).

Remarks — *Fellhanera bouteillei*, *F. emarginata* Lücking, *F. microdiscus* (Vain.) Vězda, *F. sublecanorina* (Nyl.) Vězda, and *F. semecarpi* are the other *Fellhanera* species reported from Vietnam. *Fellhanera bouteillei* and *F. semecarpi* differ in having 1-septate ascospores and light-colored apothecia; *F. emarginata* has a reduced exciple and broader ascospores with constrictions at the septa; *F. sublecanorina* has a bluish gray thallus containing isousnic acid and other substances and an apothecium with a well-developed whitish margin; *F. microdiscus* has 5-septate ascospores that are slightly curved and attenuated at one end and an apothecium with a thin but distinct margin. For further descriptions of *F. rhapidophylli,* see Lücking (2008).

Fellhanera semecarpi (Vain.) Vězda, Folia geobot. phytotax. 21: 215, 1986

Thallus dispersed into rounded patches, 3–7 mm across, smooth, greenish gray. Apothecia rounded to irregular in outline, 0.15–0.3 mm diam.; disc plane, ochraceous yellow to reddish brown; margin thin, pale gray. Excipulum

paraplectenchymatous. Hypothecium brown. Hymenium 40–50 µm high, hyaline. Asci 30–40 × 8–10 µm, 8-spored. Ascospores 1-septate, with constriction at septum, 12–15 × 4–5 µm. Pycnidia not observed.

Chemistry — Spot test reactions: thallus K–, C–, KC–, P–. Secondary metabolites: none detected.

Distribution & ecology — Pantropical (Lücking 2008); new to Vietnam. At the collection site the species was found growing on the understory leaves in lowland tropical rain forest along with *Calopadia puiggarii* and *Porina nitidula*.

Specimens Examined – **VIETNAM: Quang Binh Province**: Vườn quốc gia Phong Nha-Kẻ Bàng, 17°33′04.20″N, 106°18′03.60″E, alt. 132 m, on leaf of *Cinnamomum*, 05 February 2010, T. Nguyen 100168a, 100168c, 100168e (KoLRI).

Remarks — *Fellhanera bouteillei, F. emarginata, F. microdiscus, F. rhapidophylli* and *F. sublecanorina* are the other *Fellhanera* species reported from Vietnam (Vězda 1977, Nguyen et al. 2010). *Fellhanera emarginata, F. microdiscus, F. rhapidophylli,* and *F. sublecanorina* differ in having 3–5-septate ascospores. *Fellhanera bouteillei* has 1-septate ascospores but differs in its farinose to granulose thallus bearing secondary metabolites (usnic acid, isousnic acid, zeorin and sometimes asemone). For further descriptions of *F. semecarpi,* see Lücking (2008).

Porina subnitidula Coliín & A.B. Peña, Phyton (Horn) 44: 176, 2004

Thallus dispersed into irregular patches, brownish gray. Perithecia black, sessile, hemispherical with base spreading, 0.4–0.6 mm diam., glabrous, pure black, slightly shiny. Excipulum 20–30 µm thick, brownish black, K+ black. Involucrellum black, K–, exposed. Asci fusiform, 80–100 × 10–12 µm. Ascospores fusiform, 5-septate, without constriction at septa, 15–30 × 5–7 µm, colorless. Pycnidia not observed.

Chemistry — Spot test reactions: thallus K–, C–, KC–, P–. Secondary metabolites: none detected.

Distribution & ecology — Neotropical (Lücking 2008); new to Vietnam. At the collection site the species was found growing on the understory leaves in lowland tropical rain forest at an elevation of 780 m.

Specimen Examined – **VIETNAM: Quang Binh Province**: Vườn quốc gia Phong Nha-Kẻ Bàng, 17°28′42.10″N, 106°27′35.60″E, alt. 780 m, on leaf of *Rhaphidophora*, 06 February 2010, T. Nguyen 100080a (KoLRI).

Remarks — Many *Porina* species have been reported from Vietnam (Aptroot & Sparrius 2006). Here we compare *P. subnitidula* with those having blackish, brownish or colorless involucrellum (*P. atrocoerulea* Müll. Arg., *P. diaphana, P. nitidula*). *Porina diaphana* differs in having pale yellowish-white perithecia and colorless involucrellum; *P. nitidula* has subglobose perithecia surrounded

by white tomentum, while *P. atrocoerulea* has oblong, 7-septate ascospores. For further descriptions of *P. subnitidula,* see Lücking (2008).

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Dendrothele latenavicularis sp. nov. (*Agaricales, Basidiomycota*) from the Patagonian Andes

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Abstract — *Dendrothele latenavicularis* is described as a new species from the Patagonian Andes forest. It is closely related to other species of *Dendrothele* with navicular or arachiform basidiospores —*D. arachispora, D. cymbiformis D. navicularis, D. magnenavicularis*, and *D. pitrae*— but with distinctly broader basidiospores. A key to the previous species is included.

Key words — corticioid fungi, Patagonia, *Saxegothaea conspicua*, taxonomy

Introduction

The genus *Dendrothele* Höhn. & Litsch. comprises about 45 species (Parmasto et al. 2004), most described and exclusively recorded from the Northern Hemisphere. *Dendrothele* includes species with discoid or crustose basidiomes occurring on bark of living trees and shrubs, with only few species known from fallen wood. Microscopically, it is characterized by the abundant dendrohyphidia and crystalline deposits, an adaptation to drought and exposure, large basidia, and variably shaped basidiospores, in some cases slightly thick-walled or cyanophilous. There are several worldwide taxonomic studies of the genus (e.g., Lemke 1964, Boidin et al. 1996), but only a few that focus on the Southern Hemisphere species (Cunningham 1954, 1963; Greslebin & Rajchenberg 1998; Nakasone & Burdsall 2011).

Dendrothele is a polyphyletic genus (Goranova et al. 2003, Bodensteiner et al. 2004, Binder et al. 2005, Larsson 2007), and the generic type, *Dendrothele papillosa* Höhn. & Litsch. [= *D. griseocana* (Bres.) Bourdot & Galzin], is included in the *Lachnellaceae* Boud. (*Niaceae* Jülich) within the *Agaricales* Underw., closely related to such cyphelloid genera as *Lachnella* Fr. and *Cyphellopsis* Donk. Recently, Nakasone & Burdsall (2011) described the first navicular

basidiospores in *Dendrothele* for some species from New Zealand. Some genera of cyphelloid fungi, such as *Flagelloscypha* Donk and *Lachnella*, have navicular basidiospores, which morphologically support their phylogenetic relationship with *Dendrothele*.

As a result of some collecting trips in the Valdivian rainforest we have found growing on bark of living *Saxegothaea conspicua* Lindl. (*Podocarpaceae*), a *Dendrothele* species with broad navicular basidiospores, different from previously described species, that is herein proposed as new.

Material & methods

For light microscopic studies, samples were mounted in 3% potassium hydroxide (KOH), Melzer's reagent (IKI), and 0.1% cotton blue in 60% lactic acid to establish cyanophily of basidiospores. Line drawings were made with a camera lucida attachment. Specimens are deposited in the herbarium of the Centro de Investigación y Extensión Forestal Andino-Patagónico (Esquel, Argentina), BAFC, and SALA.

Taxonomy

Dendrothele latenavicularis Gorjón, **sp. nov.** PLATES 1–4

MYCOBANK MB 519378

Basidiomata resupinata, effusa, crustacea, leve vel rimosa, subalbida. Systema hypharum monomiticum, hyphae fibulatae, crystallis perabundantibus. Dendrophyses copiosae, graciles, leves vel incrustates. Cystidia desunt. Basidia suburniformia vel subcylindracea, cum fibula basali, 4-sterigmatibus. Basidiosporae leves, late navicularis, tenuitunicatae, hyalinae, 16–21 × 8–11 µm, inamyloideae, indextrinoideae, forte cyanophileae. Differt Dendrothele naviculari, D. magnenaviculari et D. cymbiformi basidiosporis latioribus. Ad corticem arborum coniferarum viventes.

TYPE: Argentina, Neuquén-Río Negro: Nahuel Huapi National Park, Puerto Blest (41°00′05″S 71°49′42″W), 800 m a.s.l., 30 May 2010–on bark of living *Saxegothaea conspicua*, leg. S.P.Gorjón, coll. SPG 2963. **Holotype**, BAFC; **isotypes**, SALA and herbarium of Centro de Investigación y Extensión Forestal Andino-Patagónico.

ETYMOLOGY: *latus* – Latin for broad, and *navicularis* – Latin for boat-shaped, referred to the shape of the basidiospores.

BASIDIOMATA annual, resupinate, adnate, at first orbicular, then coalescent and effused up to 3–5 cm, smooth, rimose when mature, at first whitish, turning yellowish cream with age, margin abrupt (PLATE 1). HYPHAL SYSTEM monomitic, generative hyphae with clamps, hyaline, thin-walled, straight or some tortuose, 1.5–2.5(–3) µm diam. DENDROHYPHIDIA variable, some with few branches and smooth, others richly ramified and covered by a crystalline encrustations, 1–1.5(–3) µm in diam, projecting above the basidia. CYSTIDIA absent. BASIDIA at first suburniform, cylindrical when mature, with a median constriction, thin-walled, (30–)35–45 × 10–14 µm, with 4 sterigmata, basally clamped. BASIDIOSPORES broadly navicular, tapering gradually to the vermiform distal

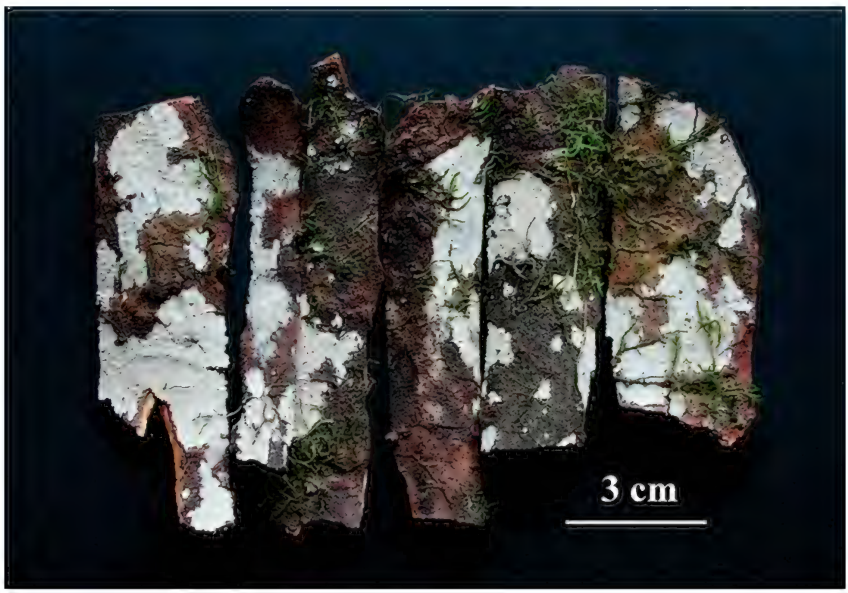

Plate 1. *Dendrothele latenavicularis*.
Basidiomes on bark (coll. S.P. Gorjón 2963, holotype)

end, with a blunt and round prominent apiculus, hyaline, smooth, thin-walled, mostly attached in groups of two or four, (16–)17–20(–21) × (8–)9–10(–11) µm, IKI–, with distinctly and strongly cyanophilic walls (plates 2–4).

Distribution and Ecology — *Dendrothele latenavicularis* is known only from the Patagonian Andes forests of Argentina (Valdivian rainforest). It grows on bark of living *Saxegothaea conspicua*, an endemic southern South American conifer, in mixed forests with *Nothofagus dombeyi* (Mirb.) Oerst., *Luma apiculata* (DC.) Burret, and *Chusquea culeou* E. Desv. If its occurrence coincides with host distribution, then it should be present in southern Chile also, although we have surveyed some areas in the closest regions (Region X and XI) with no success. In many specimens of *S. conspicua* where *D. latenavicularis* grows, it is also frequent to find associated to the bark *Globulicium hiemale* (Laurila) Hjortstam, easily to distinguish by the pinkish hymenial surface and the conspicuous white fibrillose to rhizomorphic margin.

Additional specimens examined — **ARGENTINA. Neuquén-Río Negro**: Nahuel Huapi National Park, Puerto Blest (41°00′05″S 71°49′42″W), 800 m a.s.l., 29-30 May 2010–on bark of living *Saxegothaea conspicua*, leg. S.P.Gorjón coll. SPG 2911, 2912, 2914, 2915, 2917, 2918, 2920, 2923, 2926, 2930, 2962. Idem, 12-14 April 2011, coll. *SPG 3215, 3242*. **Neuquén**: Lanin National Park, Lago Queni (40°08′35″S 71°43′05″W), 1080 m a.s.l., 18 May 2010–on bark of living *S. conspicua*, leg. S.P.Gorjón coll. SPG 2831, 2832.

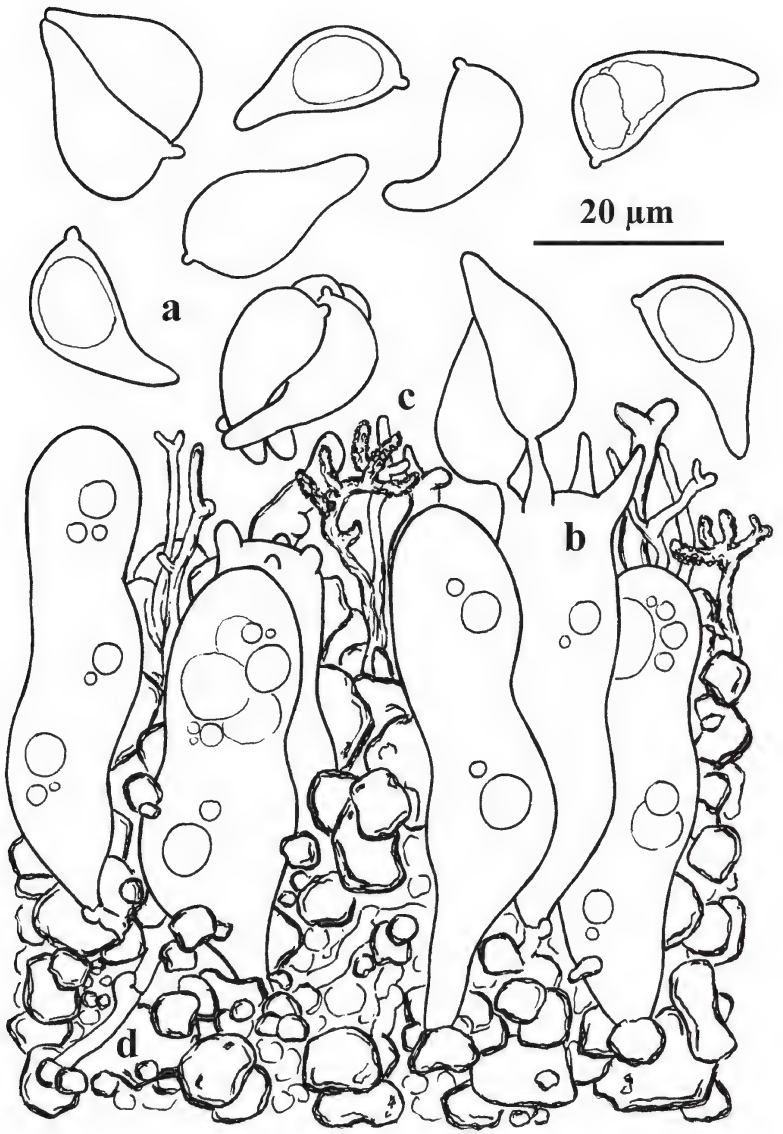

Plate 2. *Dendrothele latenavicularis*.
Hymenial elements a) basidiospores, b) basidium, c) dendrohyphidia, d) generative hyphae (coll. S.P. Gorjón 2963, holotype)

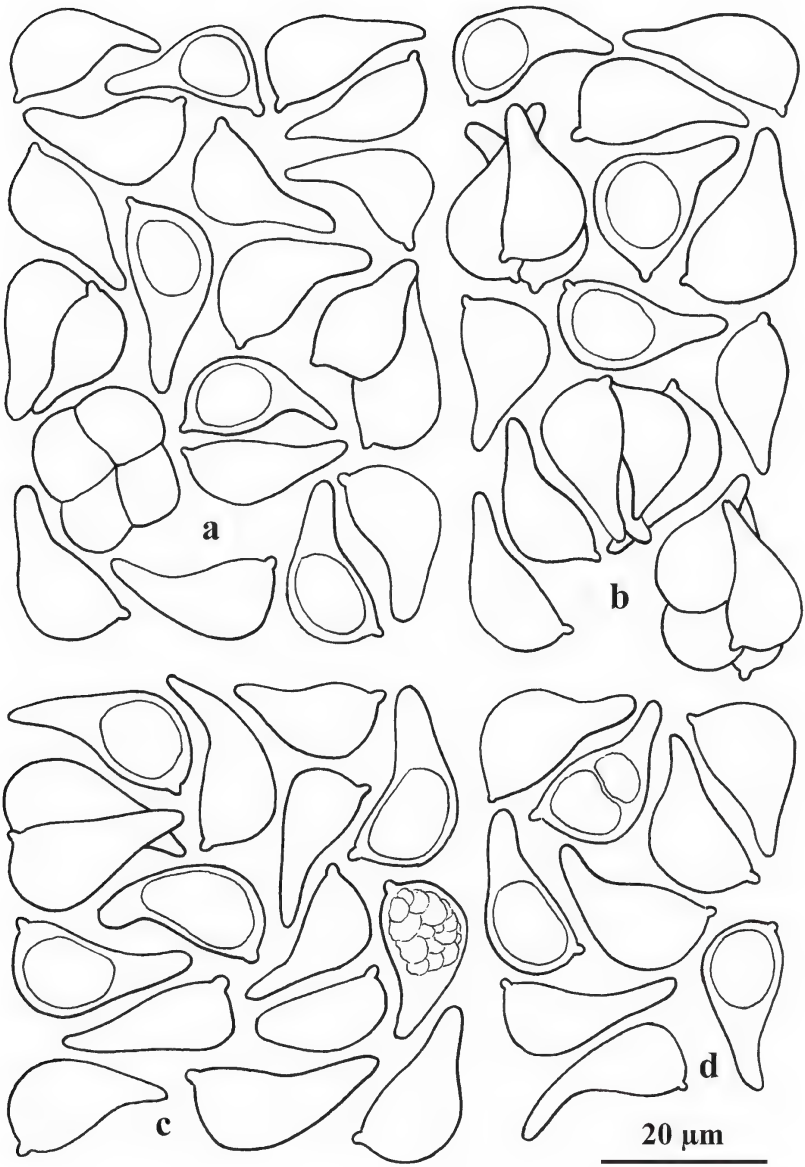

Plate 3. *Dendrothele latenavicularis*.
Basidiospores (coll. S.P. Gorjón a) 2962, b) 2920, c) 2915, d) 2918)

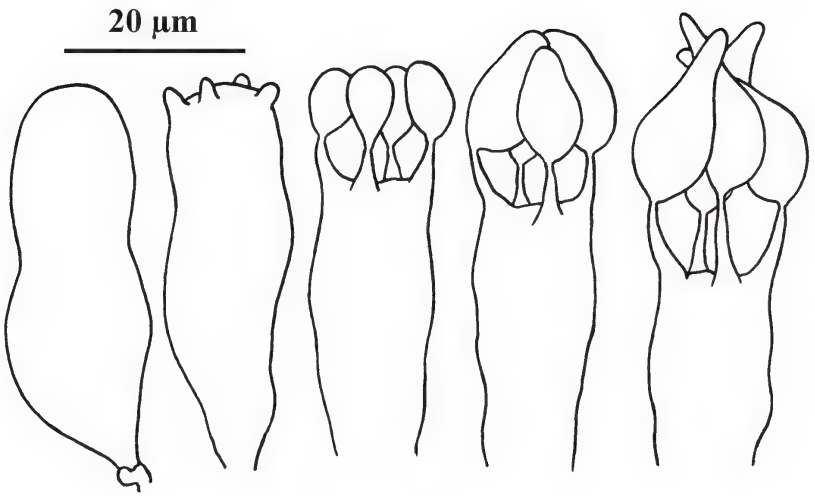

Plate 4. *Dendrothele latenavicularis*.
Basidiosporogenesis (coll. S.P. Gorjón 2963, holotype)

Comments — *Dendrothele latenavicularis* belongs to the group of *Dendrothele* species with navicular basidiospores, a spore feature recently described by Nakasone & Burdsall (2011) for three species from New Zealand. *Dendrothele navicularis* Nakasone & Burds. has irregularly subfusiform to navicular basidiospores, often medially constricted, sometimes curved, with a small, distinct apiculus, (11–)14–16(–19) × 5.5–7.2(–8) µm. In addition it has rare suburniform, pyriform, or narrowly clavate to cylindric cystidia. *Dendrothele magnenavicularis* Nakasone & Burds. produces long, narrowly navicular basidiospores (17.3–)18–22(–24) × (5.5–)6–7(–7.8) µm, with a distinct median constriction. *Dendrothele cymbiformis* Nakasone & Burds. is characterized by a hymenophore with hyphal pegs and narrowly navicular basidiospores, (11.5–)14–17.5 × 4–5(–6) µm.

Two *Dendrothele* species with arachiform basidiospores also appear to be morphologically closely related. *Dendrothele pitrae* Gresl. & Rajchenb. from Argentina, has cylindric, slightly or distinctly curved basidiospores with a median constriction, and distally obtuse or tapered, (11–)12–15(–16) × 5–5.5(–6) µm. *Dendrothele arachispora* Nakasone & Burds., from New Zealand, has ellipsoid basidiospores, (14–)16–18 × (6.5–)7.8–8.5(–9.5) µm, with a slight median constriction (peanut-shape).

Dendrothele latenavicularis differs from the species discussed above in its distinctly broader navicular basidiospores that lacks a median constriction and tapers to a narrow, vermiform distal end (Plate 3). Moreover, all these species

with navicular or arachiform basidiospores have different host preferences. Because no *Dendrothele* species with navicular or arachiform basidiospores is known from the Northern Hemisphere, it is likely that they have a common origin in the Southern Hemisphere. It would be very interesting to study these species from a molecular perspective, to determine their genetic relationships and phylogeographical patterns.

Key to *Dendrothele* species with navicular and arachiform basidiospores

1a. Basidiospores cylindric to ellipsoid with a distinct median constriction 2
1b. Basidiospores navicular to fusiform 3
2a. Basidiospores usually more than 6.5 µm wide ..... ***D. arachispora***
2b. Basidiospores narrow, usually up to 6 µm wide ..... ***D. pitrae***
3a. Basidiospores (8–)9–11 µm wide ..... ***D. latenavicularis***
3b. Basidiospores up to 6–7(–8) µm wide 4
4a. Hymenophore with hyphal pegs, basidiospores up to 6 µm wide ... ***D. cymbiformis***
4b. Hymenophore smooth, basidiospores 5.5–8 µm wide.....5
5a. Cystidia present, basidiospores 11–19 µm long ..... ***D. navicularis***
5b. Cystidia absent, basidiospores 17–24 µm long ..... ***D. magnenavicularis***

Acknowledgments

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Endocalyx melanoxanthus var. *melanoxanthus* (*Ascomycota*): new to Brazil and three new hosts

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Abstract — *Endocalyx melanoxanthus* var. *melanoxanthus* was recovered during a survey of microfungi on palms in Atlantic Rainforest areas of Northeast Brazil. Three new hosts were identified for the fungus, newly reported for Brazil.

Key words —anamorph, biodiversity, Brazilian mycota, palm fungi, taxonomy

Introduction

Brazil has one of the richest *Arecaceae* (palm family) diversity in the world, hosting at least 266 species (39 genera), of which 111 are regarded as endemic (Leitman et al. 2010). Although Farr et al. (2011) list about 100 species of microfungi associated with this family for the country, the palm mycobiota remains almost unknown. Silva & Minter (1995) cite 38 ascomycete species on palms in Pernambuco State and only six in Bahia State based on works by Chaves Batista and collaborators. During palm microfungal surveys of Pernambuco and Bahia states along the Brazilian northeastern coast (Vitoria et al. 2008, 2010, 2011; Souza et al. 2008), the fungus *Endocalyx melanoxanthus* var. *melanoxanthus* was found on *Acrocomia intumescens* Drude, *Euterpe edulis* Mart., and *E. oleracea* Mart. This report of the fungus for the first time for Brazil on three new hosts is part of an ongoing project to further knowledge of palm microfungi in the Brazilian Northeast Atlantic Rainforest.

Materials & methods

Dead palm leaves were collected in the municipalities of Igarassu, Recife, Cabo de Santo Agostinho, and Tamandaré in Pernambuco State and Uruçuca in Bahia

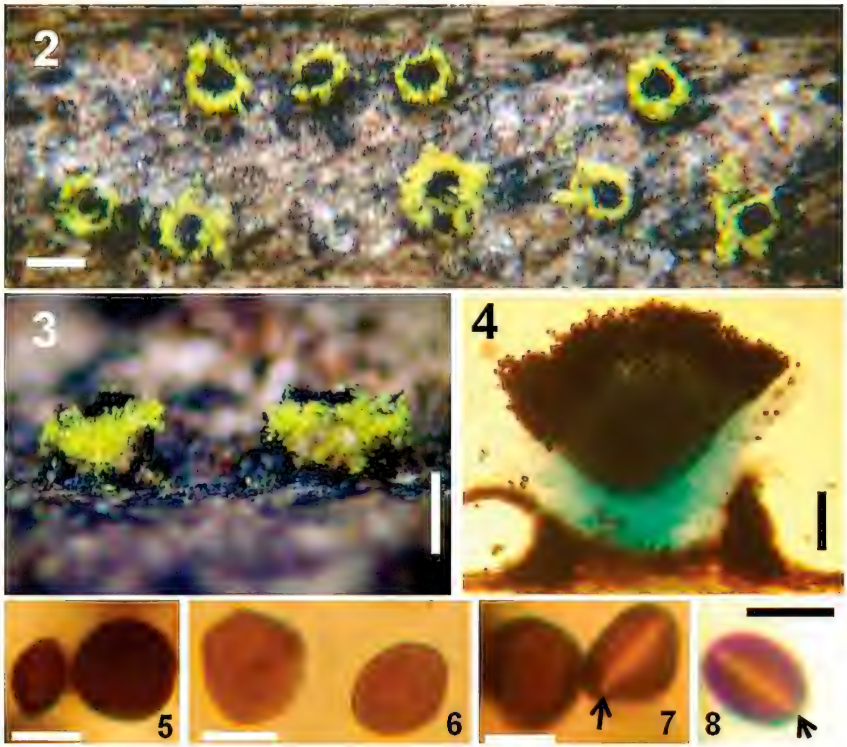
Plate 1. Map of the northeastern Brazilian coast with the collecting sites indicated: 1. Municipality of Igarassu: 07°48′56.7″S 34°57′17.3″W, 49 m; 2. Municipality of Recife: 08°00′36.9″S 34°56′57.2″W, 30 m; 3. Municipality of Cabo de Santo Agostinho: 08°14′01.0″S 35°02′49.1″W, 54 m; 4. Municipality of Tamandaré: 08°43′22.8″S 35°10′40.5″W, 109 m; 5. Municipality of Uruçuca: 14°56′132″S 39°16′601″W, 101 m.

State (Plate 1). The collected specimens were incorporated into CEPEC Herbarium (Mycological Collection) in Itabuna, Bahia. Observations under the stereomicroscope preceded study of squash preparations and vertical, free hand sections of the conidiomata. Morphological features were described, measured and photographed using a Carl Zeiss microscope. All measurements were made in water preparations. In most cases, the samples were stained with lacto-glycerol cotton blue.

Taxonomy

Endocalyx melanoxanthus (Berk. & Broome) Petch
var. *melanoxanthus* Pl. 2–8

Conidiomata 0.4–1.0 mm high, up to 0.5 mm diam, yellow, cup-shaped to cylindrical, scattered. Conidiophores up to 2 µm wide, septate, hyaline, thread-like or hyphoid. Conidiogenous cells 1–1.5 µm long, integrated, determinate, unicellular, hyaline, knob-like. Conidia 10.4–17 × 10–15 µm in face view, and



Plates 2–8. *Endocalyx melanoxanthus* var. *melanoxanthus*: 2–3. Conidiomata on host surface; 4. Section of conidioma; 5–8. Conidia (arrows indicate germ-slits). (Scale bars: 2 = 0.5 mm; 3 = 1 mm; 4 = 100 µm; 5–8 = 10 µm).

7–9 µm in side view (mean = 13.5 × 11.5 × 7.3 µm, n = 50), 1-celled, smooth-walled, flattened, round, oval or slightly polygonal in face view, at first pale, dark brown at maturity, solitary, guttulate, with a longitudinal, straight germ slit.

Specimens examined — **BRAZIL. Pernambuco:** Igarassu, Refúgio Ecológico Charles Darwin, on dead leaf (rachis) of *Acrocomia intumescens,* 18.VI. 2009, coll. Nadja Vitoria, det. JL Bezerra (CEPEC 2169); Recife, Parque Estadual Dois Irmãos, on dead leaf (rachis) of *Euterpe oleracea*, 13. VI. 2009, coll. Nadja Vitoria, det. JL Bezerra (CEPEC 2170); Cabo de Santo Agostinho, Reserva Ecológica de Gurjaú, on dead leaf (rachis) of *E. oleracea*, 09. VI. 2009, coll. Nadja Vitoria, det. JL Bezerra (CEPEC 2171); 16.XI. 2009, coll. Nadja Vitoria, det. JL Bezerra (CEPEC 2172); Tamandaré, Reserva Biológica de Saltinho, on dead leaf (rachis) of *E. oleracea*, 03. IX. 2010, coll. Nadja Vitoria, det. JL Bezerra (CEPEC 2173). **Bahia:** Uruçuca, EMARC, on dead leaf (rachis) of *E. edulis,* 18. III. 2010, coll. Nadja Vitoria, det. JL Bezerra (CEPEC 2174).

Hosts — *Arecaceae: Acrocomia* (this paper), *Archontophoenix, Borassus, Cocos, Elaeis, Euterpe* (this paper), *Livistona, Oncosperma, Phoenix, Satakentia, Trachycarpus, Washingtonia; Dipterocarpaceae: Shorea; Smilacaceae: Ripogonum*, *Smilax.*

Table 1. Synopsis of conidia of *Endocalyx* species

Taxa	Conidia	Source
E. amarkantakensis U.S. Patel et al.	5–10 × 4–5 µm, smooth walled	Patel et al. 2002
E. cinctus Petch	10–13 (–17) × 6–12 × 7–8 µm, verrucose	Okada & Tubaki 1984
E. collantesis J. Mena & Mercado	10–13.5 × 7.5–11.5 × 5–7 µm, smooth	Mena Portales & Mercado Sierra 1984
E. indicus J.N. Kapoor & Munjal	7–10 × 6–8 × 1.5–2 µm, smooth	Kapoor & Munjal 1966
E. indumentum G. Okada & Tubaki	8–12 µm diam, wall ornamented	Okada & Tubaki 1984
E. melanoxanthus [var. *melanoxanthus*]	14–19 × 12–14 × 6–7 µm	Petch 1908
E. melanoxanthus var. *melanoxanthus* (CEPEC 2169)	10.4–17 × 10-15 × 7–9 µm, smooth	This publication
E. melanoxanthus var. *melanoxanthus*	12–17 × 10–14 × 7–9 (–10) µm, smooth	Okada & Tubaki 1984
E. melanoxanthus var. *grossus* G. Okada & Tubaki	9–16 × 9–15 × 6–9 µm, verrucose	Okada & Tubaki 1984
E. thwaitesii Berk. & Broome	17–21 × 16–19 µm, verrucose	Okada & Tubaki 1984

Distribution — Australia, Brazil (this paper), China, Cuba, Ghana, Hong Kong, India, Jamaica, Japan, Malaya, New Zealand, Pakistan, Papua New Guinea, Philippines, Sabah, Sarawak, Seychelles, Sierra Leone, Singapore, Sri Lanka, Taiwan, USA.

Comments — The genus *Endocalyx* includes seven species, one of which is divided into two varieties (Table 1). According to Okada & Tubaki (1984), *Endocalyx* species colonize palm litter or (rarely) vine and lilies. Our report represents the first record of *E. melanoxanthus* for Brazil, and *Acrocomia intumescens, Euterpe edulis,* and *E. oleracea* represent new hosts for the species.

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New records of rust fungi on sedges (*Cyperaceae*) from Pakistan

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Abstract —*Puccinia caricis-kouriyamensis* on *Carex karoi*, *P. caricis-pocilliformis* on *Carex* sp., *P. extensicola* var. *linosyridis-caricis* on *Carex divulsa*, and *P. conclusa* on *Cyperus difformis* (all three new to the country) and *P. conclusa* on *Cyperus difformis* were recently recorded during a survey of rust fungi on sedges in Pakistan. These rust species have the potential to serve as biological agents to control weeds and increase agricultural productivity. *Carex karoi* and *C. divulsa* are newly reported hosts for rust fungi in Pakistan.

Key words — Kaghan valley, Khyber-Pakhtoonkhwa, Punjab, *Uredinales*

Introduction

The *Cyperaceae* is a cosmopolitan family with 106 genera and ca. 5400 species (Govaerts et al. 2007) worldwide with 22 genera and 179 species known in Pakistan (Kukkonen 2001). Members of the *Cyperaceae* commonly called sedges are primarily grass-like herbs with linear leaves and parallel venation. The most important cyperaceous weeds in terms of their adverse effect on agriculture include *Cyperus difformis*, *C. esculentus*, *C. iria*, *C. rotundus*, and *Fimbristylis miliacea* (Bryson & Carter 2008). Among the important fungal pathogens of sedges are rust fungi (*Basidiomycota*: *Uredinales*). Previously, thirteen species of *Cyperaceae* have been reported as hosts for rust fungi from Pakistan (Ahmad et al. 1997; Afshan & Khalid 2008, 2009; Afshan et al. 2009). In the present study, four *Puccinia* taxa, three of them new records for Pakistan, are recorded on four cyperaceous hosts.

Materials & methods

During the survey of rust fungi from Pakistan, infected plants were collected from different areas of Pakistan. Healthy plants were collected along with inflorescences and fruits for accurate identification. Host plants were identified by comparing them with specimens in the herbarium of the Department of Botany, University of the Punjab, Lahore (LAH).

Free hand sections of infected portions of material and spores were mounted in lactophenol. Semi-permanent slides were prepared by cementing cover slips with nail lacquer (Dade & Gunnell 1969). Preparations were observed under a NIKON YS 100 microscope. Drawings of spores were made by using a Camera Lucida (Ernst Leitz, Wetzlar, Germany). Spores were measured using an ocular micrometer (Zeiss, St Albans, Hertfordshire, England). At least twenty-five spores were measured for each spore stage. Measurements include the usual range and the arithmetic means; extremes are given in parentheses.

Enumeration of Taxa

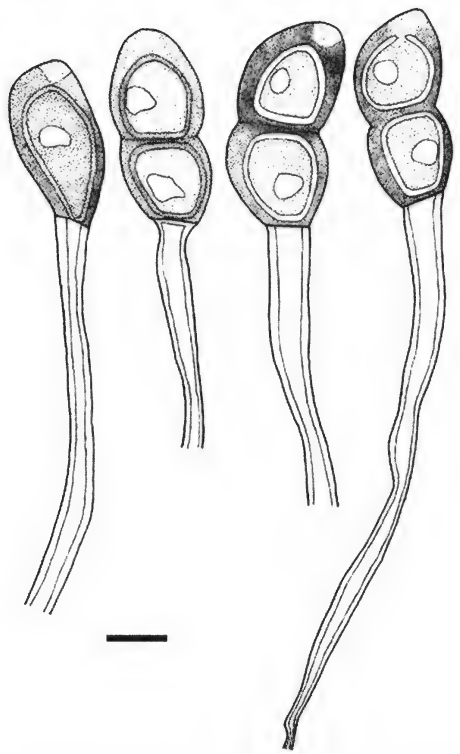

FIG. 1. Lucida drawings of 1–2 celled teliospores of *Puccinia caricis-kouriyamensis*. Scale bar = 15 µm.

Puccinia caricis-kouriyamensis Morim., J. Jap. Bot. 47(4): 117 (1972). FIG. 1

SPERMOGONIA, AECIA and UREDINIA not seen. TELIA amphigenous, naked, black, scattered, compact, rounded, erumpent, pulverulent, 140–280 × 240–440 µm. TELIOSPORES ellipsoid or oblong, pale brown to chestnut brown, constricted at septum, attenuate or rounded at base, 19–27 × 39–59(–63) µm (mean 22 × 50.8 µm); wall smooth, two-layered, pale brown to chestnut brown,

1.8–4.7 µm; germ pores two, one per cell, apical in upper cell, obscure in lower cell, most probably adjacent to septum; apex rounded or conical, dark brown with hyaline region, 4–9 µm thick; pedicel persistent, hyaline, 5–10 µm wide, 26–118 µm long. Mesospores intermixed, pale brown, sometimes chestnut brown, clavate to oblong, attenuated below, 14–24 × 31–53 µm (mean 18 × 40.8 µm); wall pale brown to dark brown, 1–2.4 µm, smooth; apex conical, pale brown to dark brown with hyaline region, 4–10 µm thick; pedicel hyaline, persistent, up to 73 µm long.

Material examined: **PAKISTAN, Gilgit Baltistan**, on *Carex karoi* Freyn, 26 July 2010, coll. & det. Malka Saba 01, with III stage (LAH 1140).

Rust fungi previously reported on *Carex* spp. from Pakistan include *Puccinia caricina* DC. and *P. caricis-filicinae* Barclay on *Carex filicina* Nees, *P. dioicae* Magnus and *P. pakistani* S. Ahmad on *Carex nubigena* D. Don, *P. bolleyana* Sacc. on *Carex flacca* Schreb., and *P. subepidermalis* Afshan et al. on *Carex curta* Gooden. (Ahmad et al. 1997, Afshan & Khalid 2009, Afshan et al. 2009).

Puccinia caricis-kouriyamensis, first reported on *Carex brunnea* Thunb. (kogome-suge) from Japan (Hiratsuka et al. 1992), is a new record for Pakistan. *Carex karoi* is a newly recorded host.

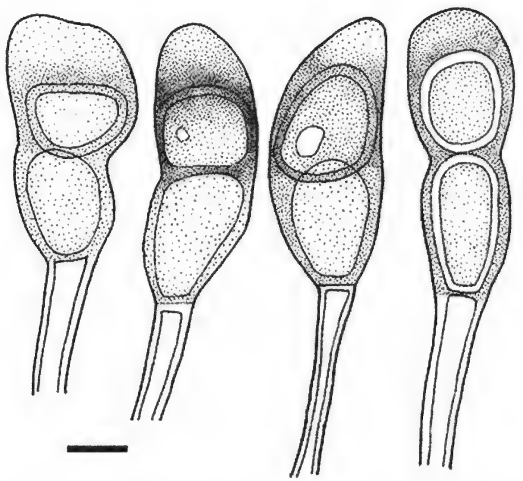

Fig. 2. Lucida drawings of teliospores of *Puccinia caricis-pocilliformis*. Scale bar = 10 µm.

Puccinia caricis-pocilliformis Morim., J. Jap. Bot. 49(8): 228 (1974). Fig. 2

Spermogonia, aecia and uredinia not seen. Telia on abaxial side of leaf, black, erumpent, scattered, first covered by epidermis, soon naked, compact, 80–200 × 100–600 µm. Teliospores clavate, obovate-clavate or oblong-clavate, yellowish brown to dark brown, constricted at septum, attenuated below,

(15–)18–24(–28) × (32–)37–60(–74) µm (mean 21.2 × 48.4 µm); wall smooth, yellowish brown or paler, two-layered, 1.5–2 µm; apex rounded, conical or obliquely conical, rarely truncate, dark brown, 6–17 µm thick; pedicel long, persistent, hyaline to pale yellow, 4–9.4 × (4–)7–71 µm. Mesospores present, clavate, pale brown, attenuated below, 13–15 × 36–47 µm (mean 13.6 × 39 µm); apex conical, 2–9 µm thick; pedicel short, up to 12 µm long.

Material examined: **PAKISTAN, Gilgit Baltistan**, on *Carex* L., 26 July 2010, coll. & det. Malka Saba 02, with III stage (LAH 1141).

Puccinia caricis-pocilliformis, first reported on *Carex tristachya* var. *pocilliformis* (Boott) Kük. (kotsubu-moegisuge) from Japan (Hiratsuka et al. 1992) , is a new record for Pakistan.

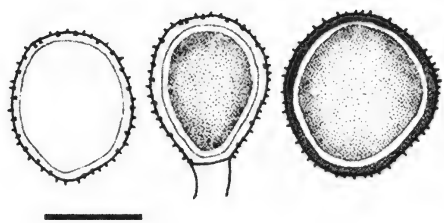

Fig. 3. Lucida drawings of urediniospores of *Puccinia conclusa*, showing echinulate ornamentation.
Scale bar = 10 µm.

Puccinia conclusa Thüm., J. Sci. math. phys. nat. Lisboa, 1 Ser. 6(24): 237 (1878). Figs. 3–4

Spermogonia and aecia unknown. Uredinia amphigenous, mostly on abaxial side, brown, scattered, somewhat aggregated, erumpent, naked, 100–250 × 100–400 µm. Urediniospores subglobose, ellipsoid or obovoid, pale brown, 18–26 × 21–35 µm (mean 20.9 × 27 µm); wall pale brown, echinulate, 1–2(2.5–) µm; germ pores 2, equatorial; paraphyses absent. Telia amphigenous, black, loculate, covered with epidermis, aggregated, in groups, 90–110 × 90–170 µm. Teliospores cinnamon brown, clavate or fusiform, constricted at septum, attenuated below, 16–21 × 43–61 µm (mean 18.3 × 50 µm); wall cinnamon brown, smooth, 1–1.4 µm; apex rounded or conical, cinnamon brown to dark brown, 3–6 µm thick; pedicel persistent, pale brown, 6–9 × 8–41 µm.

Material examined: **PAKISTAN, Punjab**, Lahore, at 216 m a.s.l., *Cyperus difformis* L., 6 October 2010, coll. & det. Malka Saba 03, with II + III stages (LAH 1142).

Puccinia species previously reported on *Cyperus* in Pakistan include *P. cypericola* S. Ahmad and *P. philippinensis* Syd. & P. Syd. on *C. rotundus* L., *P. conclusa* [including misdeterminations as *P. romagnoliana* Maire & Sacc.] on *C. difformis* and *C. rotundus* from Chuharkana (Sheikhupura) and Karachi and on *C. iria* L.

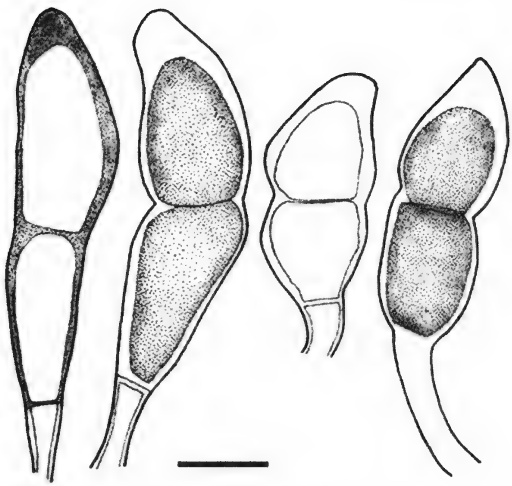

Fig. 4. Lucida drawings of teliospores of *Puccinia conclusa*. Scale bar = 10 µm.

from Lahore, and *P. cyperi-laevigati* Afshan & Khalid on *C. laevigatus* L. from Khanspur (NWFP) (Ahmad 1956a,b; Ghaffar & Kafi 1968, Hasnain et al. 1959, Afshan & Khalid 2008, Afshan et al. 2008).

Although *Puccinia conclusa* has been previously reported from other Pakistan localities, the Lahore collection is the first found on *Cyperus difformis,* a new host.

Puccinia extensicola* var. *linosyridis-caricis (E. Fisch.) Zwetko, Biblthca Mycol. 153: 109 (1993) . Fig. 5

Spermogonia and aecia not seen. Uredinia amphigenous, scattered in rows, golden brown, naked, covered with epidermis, 70–100 × 100–300 µm. Urediniospores sub-globose, ovoid or ellipsoid, pale brown, 16–24 × 20–27 µm (mean 20.5 × 23.4 µm); wall pale brown, echinulate, 1–2(–2.5) µm; germ pores 2, supraequatorial; pedicel hyaline, deciduous, 6–18 × 18–29 µm. Telia on abaxial side, dark brown to blackish brown, scattered in rows, naked, erumpent, 95–150 × 100–300 µm. Teliospores ellipsoid or oblong, cinnamon brown to dark brown, 16–26 × 39–64 µm (mean 21.5 × 51.4 µm), constricted at septum, attenuated at base; wall smooth, 1.5–3 µm; apex rounded, conical or sometimes truncated, dark brown, 8–15 µm; pedicel hyaline to pale brown, persistent, 5–13 × 15–41 µm.

Material examined: **PAKISTAN, Khyber-Pakhtoonkhwa**, Kaghan valley, Jalkhud, 3300 m a. s. l., *Carex divulsa* Stokes, 16 August 2009, coll. & det. Malka Saba 04, with II + III stages (LAH 1143).

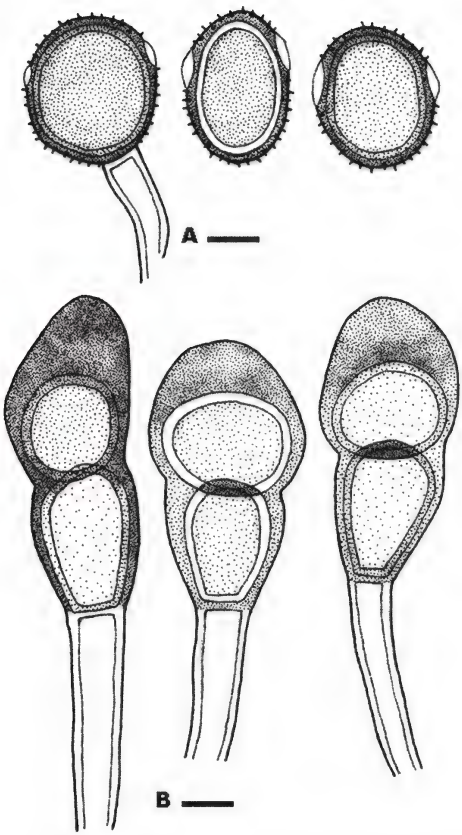

Fig. 5. Lucida drawings of *Puccinia extensicola* var. *linosyridis-caricis*.
(A) Urediniospores showing echinulate ornamentation and supraequatorial germ pores.
(B) Teliospores. Scale bar = 10 µm.

Puccinia extensicola var. *linosyridis-caricis*, first reported on *Carex humilis* Leyss. from Japan (Hiratsuka et al. 1992), is a new record for Pakistan, and *Carex divulsa* is a newly recorded host.

Key to the rust fungi on *Cyperaceae* in Pakistan

On *Carex*

1. Telia amphigenous 2
1. Telia hypophyllous, rarely amphigenous 4
2. Largest teliospore <50 µm long, oblong or oblong to clavate; teliospore apex 8–14 µm thick ..... *Puccinia caricis-filicinae*
2. Largest teliospore >50 µm long 3

3. Teliospore pedicel short, <10 µm; teliospore apex 4–6 µm thick; uredinial paraphyses clavate to capitate ..... *P. subepidermalis*
3. Teliospore pedicel long, >10 µm; teliospore apex 4–9 µm thick; uredinial paraphyses absent ..... *P. caricis-kouriyamensis*
4. Largest teliospore ≤60 µm long 5
4. Largest teliospore >60 µm long 7
5. Teliospore pedicel short, ≤10 µm ..... *P. pakistani*
5. Teliospore pedicel long, >10 µm 6
6. Teliospores clavate to oblong, 21–24 µm wide; urediniospore germ pores 2, equatorial ..... *P. bolleyana*
6. Teliospores clavate, 16–24 µm wide; urediniospore germ pores 2, supraequatorial ..... *P. dioicae*
7. Teliospore pedicel <50 µm long 8
7. Teliospore pedicel >50 µm long; teliospores obovate-clavate or oblong-clavate; apex ≤17 µm thick ..... *P. caricis-pocilliformis*
8. Urediniospore germ pores 3–4, equatorial ..... *P.caricina*
8. Urediniospore germ pores 2, supraequatorial. ... *P. extensicola* var. *linosyridis-caricis*

On *Cyperus*

1. Urediniospore germ pores two, equatorial 2
1. Urediniospore germ pores >two, scattered; paraphyses clavate; teliospores 10–18 × (31–) 34–45 µm ..... *P. cyperi-laevigati*
2. Largest teliospore >50 µm long 3
2. Largest teliospore ≤50 µm long; teliospore apex ≤7 µm long; urediniospores 14–18 × 14–19 µm ..... *P. philippinensis*
3. Teliospore apex <10 µm thick; urediniospores 18–26 × 21–35 µm ..... *P. conclusa*
3. Teliospore apex >10 µm thick; urediniospores 15–20 × 20–25 µm ..... *P. cypericola*

On *Fimbristylis*

Urediniospores 11–18 × 18–23 µm, germ pores 3, supraequatorial; teliospores long ellipsoid or fusoid, 10–18 × 35–55 µm ..... *P. flavipes* Syd. & P. Syd.

On *Scirpus*

1. Teliospores two–celled; urediniospores 12–24 × 19–32 µm ..... *P. scirpi* DC.
1. Teliospores one-celled; urediniospores 18–24 × 24–34 µm ..... *Uromyces lineolatus* (Desm.) J. Schröt.

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A new species and new records of *Endophragmiella* from China

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Abstract— Four *Endophragmiella* species are described and illustrated: *E. nanlingensis* occurring on dead branches of *Melicope triphylla* is proposed as new, and *E. pulchra, E. rostrata,* and *E. resinae* found on dead branches of unidentified plants are recorded for the first time from China. The specimens are deposited in Herbarium of Shandong Agricultural University, Plant Pathology (HSAUP) and Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS).

Key words—anamorphic fungi, hyphomycete, taxonomy

Sutton (1973) proposed the genus *Endophragmiella* to accommodate two species, *E. pallescens* B. Sutton and *E. canadensis* (Ellis & Everh.) B. Sutton. Subsequently, Hughes (1979) emended the genus and gave a very detailed account of conidiogenesis and generic concepts. *Endophragmiella* is characterized by macronematous, mononematous conidiophores with integrated, percurrently proliferating conidiogenous cells and acrogenous, solitary, septate conidia with rhexolytic secession. Presently, more than 80 species are accepted in *Endophragmiella*, most of which are reported from rotten wood, dead branches, and decaying leaves of various plants; a few species have associations with other fungi (Sutton 1973, Hughes 1979).

During a continuing survey of saprobic fungi in tropical forests of Guangdong Province, China, four *Endophragmiella* species were found on dead branches. *Endophragmiella nanlingensis* is proposed herein as new, based on its distinctive conidial characters, and *E. pulchra, E. rostrata* and *E. resinae* are recorded from China for the first time.

Endophragmiella nanlingensis S.C. Ren & X.G. Zhang, **sp. nov.** Fig. 1

MycoBank MB561001

Coloniae in substrato naturali effusae, pilosae, fuscae ad usque atrae. Mycelium immersum, sparsum, ex hyphis septatis, laevibus, pallide brunneis, ramosis, 2–4 µm latis

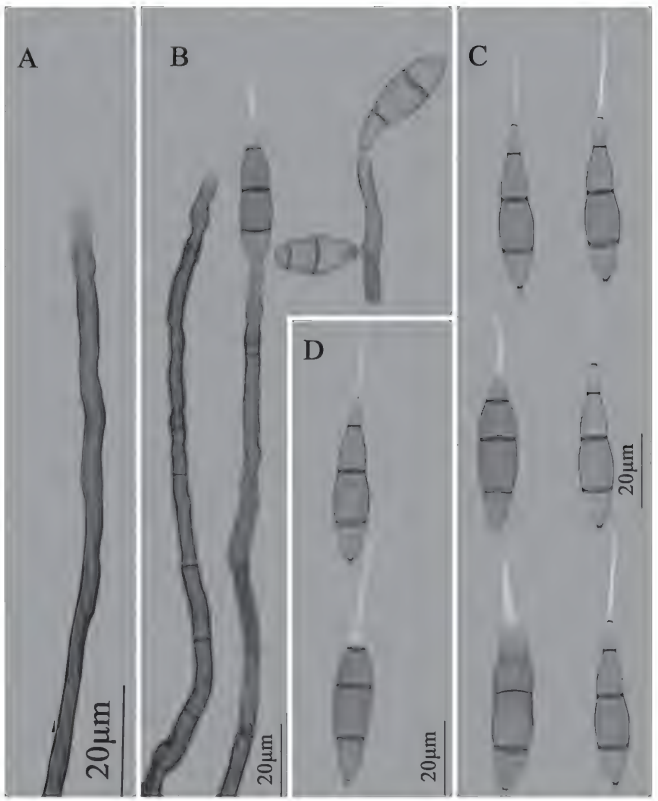

Fig. 1. *Endophragmiella nanlingensis*. A. Conidiophore and conidiogenous cell. B. Conidiophores, conidiogenous cells and conidia. C–D. Conidia.

compositum. Conidiophora *macronematosa, mononematosa, singularia vel fasciculata, simplicia, erecta, recta vel leviter flexuosa, laevia, septata, brunnea, apicem versus pallidiora, 80–160 μm alta, 4.5–9 μm lata, interdum ad basim inflata, 1–5 proliferationes percurrentes elongantia.* Cellulae conidiogenae *monoblasticae, integratae, terminales, percurrentes, cylindricae, ad apicem contractae et truncatae.* Conidia *acrogena, solitaria, late fusiformia, rostrata, (3–)4(–5)-euseptata, ad septa interdum leviter constricta, cum cellula centrali brunnea, cellula basali et apicali pallide brunnea, laevia, 55–80 μm longa, 10–13 μm lata, cum rostro 23–33 μm longo, ad basim 1.5–2 μm, ad fundamentum distincta fractam ob partem cellulae conidiogenae superiorem fimbriata.*

Holotype: **CHINA. Gungdong Province**: tropical forest of Nanling, on dead branches of *Melicope triphylla* (Lam.) Merr. (*Rutaceae*), 10 Dec. 2010, Sh.C. Ren, HSAUP H8334 (isotype HMAS 146104).

Etymology: named for the collection locality.

Colonies on natural substratum effuse, hairy, dark blackish brown to black. Mycelium immersed, sparse, composed of septate, smooth, pale

brown, branched hyphae 2–4 μm wide. Conidiophores macronematous, mononematous, arising singly or sometimes fasciculate, simple, erect, straight or slightly flexuous, smooth, septate, brown, paler towards the apex, 80–160 μm high, 4.5–9 μm wide, sometimes swollen at the base, with 1–5 percurrent proliferations. Conidiogenous cells monoblastic, integrated, terminal, percurrent, cylindrical, tapered to a truncate apex. Conidia acrogenous, solitary, broadly fusiform, rostrate, (3–)4(–5)-euseptate, slightly constricted at the septa, central cells brown, basal cells and apical cells pale brown, smooth, 55–80 μm long, 10–13 μm wide, rostrum 23–33 μm long, base truncate, 1.5–2 μm wide, with a distinct basal frill of 2.5–3.5 μm long, derived from the apex of the conidiogenous cell.

Notes: *Endophragmiella nanlingensis* is closely related to *E. fusiformis* W.P. Wu (Wu & Zhuang 2005) in its proliferating conidiophores producing fusiform, rostrate, multiseptate conidia, except that *E. fusiformis* produces conidia that are narrower (7.5–9 μm) and have more (6–7) septa. In addition, the conidia of *E. nanlingensis* slightly constricted at the septa, while those in *E. fusiformis* are not.

Endophragmiella rostrata (Kirk 1985) and *E. variabilis* R.F. Castañeda (Castañeda Ruíz 1988) are also morphologically similar to *E. nanlingensis*. However, *E. rostrata* conidia are obclavate, smaller (13–24 × 4–5 μm), and have fewer (3) septa, while *E. variabilis* produces obclavate or Y-shaped, smaller conidia (15–23 × 4–5 μm, ramuli 9–10 × 3 μm) with fewer septa (2–4-septate).

Endophragmiella pulchra (B. Sutton & Hodges) P.M. Kirk. Trans. Br. Mycol. Soc. 78: 298 (1982) Fig. 2

Anamorphic fungi. Colonies effuse, blackish brown to black, hairy. Mycelium immersed in the substratum, composed of branched, septate, pale brown hyphae. Conidiophores macronematous, mononematous, arising singly or in a group, erect, straight or slightly flexuous, sometimes swollen at the base, smooth, brown, paler towards the apex, septate, 120–400 μm long, 4–7 μm wide, with up to 3 or more percurrent proliferations at the apex. Conidiogenous cells monoblastic, integrated, terminal, percurrent, cylindrical. Conidia holoblastic, terminal, solitary, dry, smooth, ellipsoid, rostrate, 3-septate, sometimes slightly constricted at the septa, median cells brown, basal and apical cell pale brown, conidia 35–40 μm long (including rostrum), 5.5–6.5 μm wide, rostrum 8–11 μm long, with a small basal frill derived from the apex of the conidiogenous cell.

Specimens examined: **China. Gungdong Province**: tropical forest of Nanling, on dead branches of unidentified plant, 10 Dec 2010, Sh.C. Ren, HSAUP H8423; **Fujian Province**: forest park of Wuyishan, on dead branches of unidentified plant, 8 Aug. 2010, Y.D. Zhang, HSAUP H3101 (duplicate HMAS 146105).

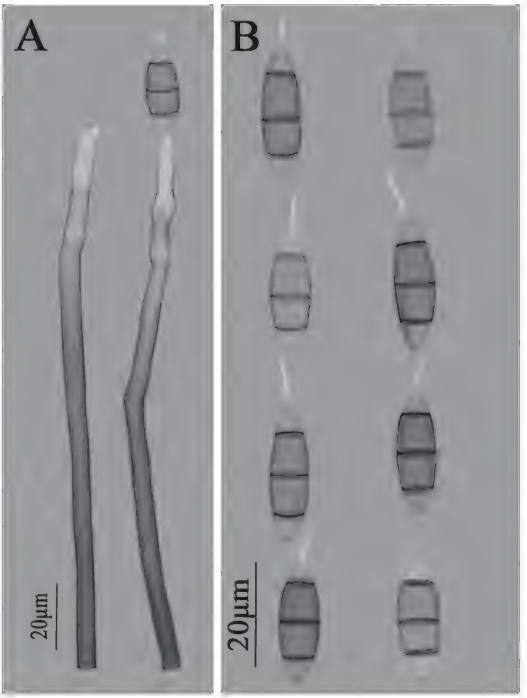

Fig. 2. *Endophragmiella pulchra.*
A. Conidiophores, conidiogenous cells and conidium. B. Conidia.

Notes: *Endophragmiella pulchra* was originally described by Sutton & Hodges (1978) as *Chaetendophragmiopsis pulchra*. Kirk (1982) subsequently transferred it to *Endophragmiella* based on its percurrently proliferating conidiogenous cells and rhexolytically seceding conidia. The conidial features in our specimen agreed well with those reported by Kirk. The species differs from others *Endophragmiella* species by its ellipsoid, 3–4-septate conidia with a filiform rostrum.

Endophragmiella rostrata P.M. Kirk, Mycotaxon 23: 325 (1985) Fig. 3

Anamorphic fungi. Colonies effuse, blackish brown to black, hairy. Mycelium mostly immersed in the substratum, composed of branched, septate, pale brown to brown, smooth-walled hyphae, 1.5–3 μm wide. Conidiophores macronematous, mononematous, arising singly or in group, erect, straight or slightly flexuous, sometimes swollen at the base, smooth, brown, paler towards the apex, septate, 120–180 μm long, 3–4 μm wide, with up to 4 or more percurrent proliferations at the apex. Conidiogenous cells monoblastic,

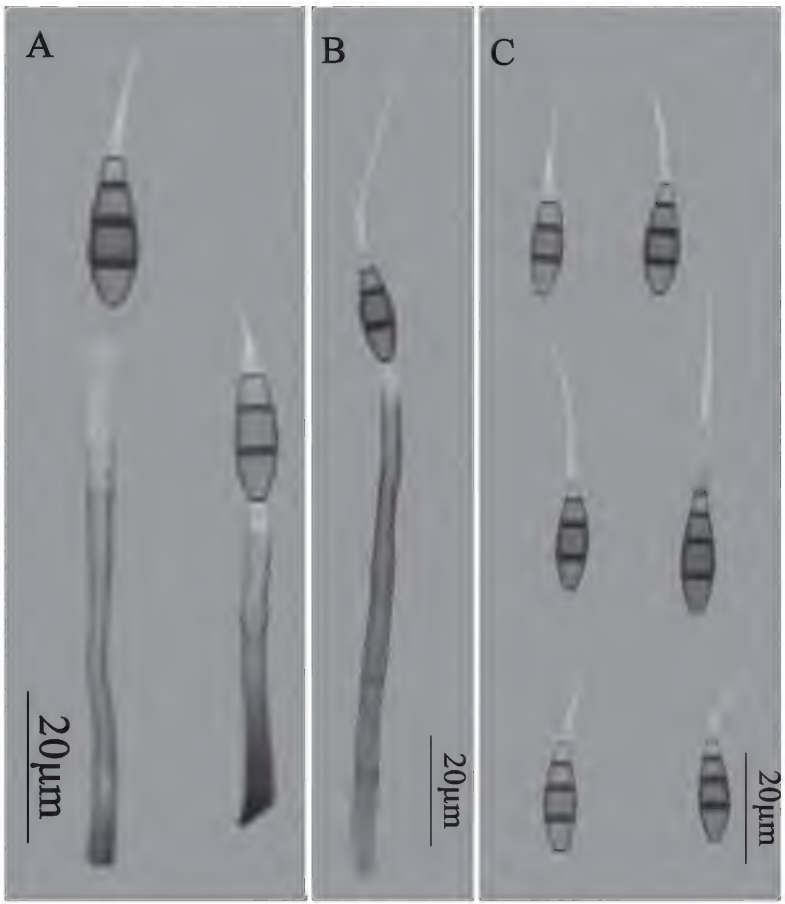

Fig. 3. *Endophragmiella rostrata*.
A–B. Conidiophores, conidiogenous cells and conidia. C. Conidia.

integrated, terminal, percurrent, cylindrical. Conidia holoblastic, terminal, solitary, dry, smooth, obclavate, rostrate, (3–)4-septate, sometimes slightly constricted at the septa, pale brown to brown, body of conidium 20–30 μm long, 4–5 μm wide, rostrum 25–40 μm long, with a small basal frill derived from the apex of the conidiogenous cell.

Specimen examined: **CHINA. Gungdong Province**: tropical forest of Nanling, on dead branches of unidentified plant, 10 Dec 2010, Sh.C. Ren, HSAUP H8226 (duplicate HMAS 146106).

Notes: Compared with the type specimen described by Kirk (1985), the conidia of our collection are slightly shorter and have more septa than those of type

material (40–85 μm long, 2–3-septate). Despite these minor differences, we believe they represent the same species. *Endophragmiella rostrata* bears some affinities with *E. corticola* P.M. Kirk (Kirk 1982) in conidial morphology, but *E. corticola* can be separated from the former by its narrowly obclavate to broadly fusiform conidia with more variable septation (1–3(–4)-septate), more variable conidial dimensions (1-septate conidia 14–23 × 5–6 μm, 2-septate conidia 18–38 × 5.5–6(–6.5) μm, 3-septate conidia 25–42 × 5.5–6.5 μm), and shorter apical cell measuring 8–12 μm long.

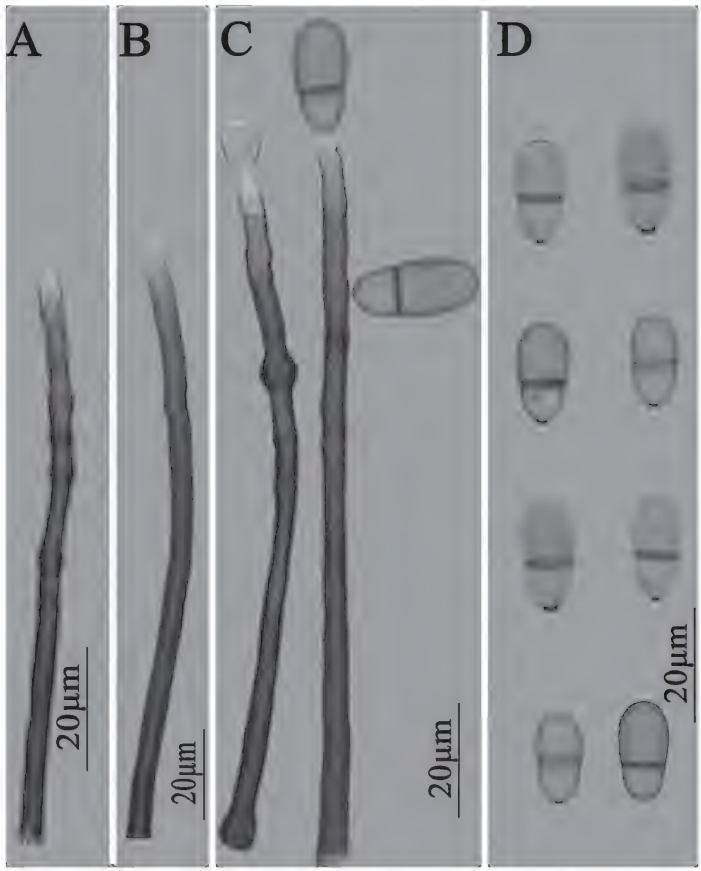

Fig. 4. *Endophragmiella resinae.*
A–B. Conidiophores. C. Conidiophores, conidiogenous cells and conidia. D. Conidia.

Endophragmiella resinae P.M. Kirk. Trans. Br. Mycol. Soc. 76: 78 (1981) Fig. 4

Colonies effuse, hairy, dark blackish brown to black, inconspicuous. Mycelium immersed in the substratum, composed of septate, smooth, pale

brown, branched hyphae 2–4 µm wide. Conidiophores macronematous, mononematous, arising singly, unbranched, erect, straight or flexuous, septate, smooth, dark brown, paler towards the apex, percurrently regenerating, 145–210 µm long, 3.5–4.5 µm wide, 7–10 µm wide at the base, with 1–6 percurrent proliferations. Conidiogenous cells monoblastic, integrated, terminal, percurrent, cylindrical, tapered to a truncate apex. Conidia holoblastic, terminal, solitary, dry, smooth, obovoid to pyriform, thick-walled, 1-septate, the upper cell longer than the basal one, basal cell pale brown, apical cell brown, 15–19 µm long, 8–9 µm wide, with a distinct basal frill of 0.5–1µm long, derived from the distal end of the conidiogenous cell.

Specimen examined: **CHINA. Gungdong Province**: tropical forest of Nanling, on dead branches of unidentified plant, 10 Dec 2010, Sh.C. Ren, HSAUP H8346 (duplicate HMAS 146107).

Notes: *Endophragmiella resinae* is similar to a group of species with obpyriform to subglobose and 1-septate conidia, such as *E. angustispora* S. Hughes, *E. bukkensis* Révay, *E. boewei* (J.L. Crane) S. Hughes, *E. bogoriensis* Rifai, *E. cambrensis* M.B. Ellis, *E. globulosa* (B. Sutton) S. Hughes, *E. pinicola* (M.B. Ellis) S. Hughes, *E. ramificata* Hol.-Jech., *E. tuberculata* S.M. Leão & Gusmão, and *E. uniseptata* (M.B. Ellis) S. Hughes (Ellis 1976, Holubová-Jechová 1986, Hughes 1978, 1979; Leão-Ferreira & Gusmão 2010, Révay 1987, Rifai 2008). *Endophragmiella resinae* can be distinguished from most of these species by its obovoid to pyriform conidia with an upper cell that is 1.5–3 times longer than the lower. *Endophragmiella globulosa,* which has some morphological similarities to *E. resinae*, differs in its subglobose to broadly obovoid conidia with a brown to dark brown upper cell and pale brown to brown basal cell. The morphological features of our specimen overlap with the type specimen as described by Kirk (1981).

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Two new *Minimelanolocus* species from southern China

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Abstract — *Minimelanolocus linderae* sp. nov. and *M. mori* sp. nov. were discovered from dead branches in southern China. They are described, illustrated, and compared with closely related taxa. The specimens are deposited in Herbarium of Shandong Agricultural University, Plant Pathology (HSAUP) and Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS).

Key words — anamorphic fungi, taxonomy

During ongoing surveys of tropical and subtropical microfungi from the forests in southern China, two interesting fungi with euseptate conidia and polyblastic conidiogenous cells were collected on dead branches of *Lindera communis* and *Morus alba*. The morphologies of their conidia and conidiogenous cells suggested they are new species of the genus *Minimelanolocus*.

Minimelanolocus linderae Jian Ma & X.G. Zhang, **sp. nov.** Fig. 1

MycoBank MB 561233

Fungus anamorphicus. Coloniae in substrato naturali effusae, brunneae vel atrobrunneae, pilosae. Mycelium partim superficiale, partim immersum in substrato, ex hyphis ramosis, septatis, pallide brunneis vel brunneis, laevibus, 1–2 µm crassis compositum. Conidiophora macronematosa, mononematosa, singula, interdum caespitosa, nonramosa, erecta, recta vel flexuosa, septata, laevia, brunnea, apice versus pallidiora, 83–133 µm longa, 2–4.5 µm crassa. Cellulae conidiogenae holoblasticae, polyblasticae, in conidiophoris incorporatae, indeterminatae, sympodialiter extendentes, terminales deinde intercalares, pallide brunneae. Loci conidiogeno inconspicuo vel leviter prominentibus, refractivi. Conidiorum secessio schizolytica. Conidia solitaria, acropleurogena, ellipsoidea vel cylindrica, basi truncata, pallide brunnea, cellulis extimis subhyalinis, laevia, plerumque 4-euseptata, raro 3-euseptata, 26–35 µm longa, 7–9 µm crassa, basi truncata 1–1.5 µm lata.

Type: China, Fujian Province: Mount Wuyi, on dead branches of *Lindera communis* Hemsl. (*Lauraceae*), 15 Aug. 2009, J. Ma, (**Holotype** HSAUP H5113; isotype HMAS146122).

Etymology: in reference to the host genus, *Lindera*.

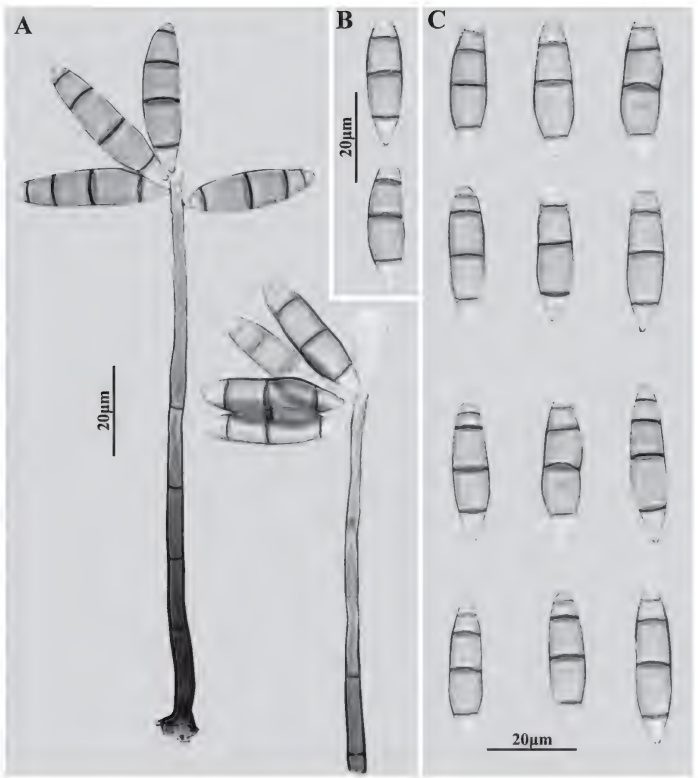

Fig. 1. *Minimelanolocus linderae*. A. Conidiophores with conidia. B, C. Conidia.

Anamorphic fungi. Colonies effused on natural substrate, brown to dark brown, hairy. Mycelium partly superficial, partly immersed, composed of branched, septate, pale brown to brown, smooth-walled hyphae, 1–2 μm thick. Conidiophores differentiated, single, sometimes caespitose, unbranched, erect, straight or flexuous, septate, smooth, brown, paler toward the apex, 83-133 μm long, 2–4.5 μm thick. Conidiogenous cells holoblastic, polyblastic, integrated, indeterminate, sympodially extending, terminal becoming intercalary, pale brown. Conidiogenous loci inconspicuous or slightly prominent, refractive. Conidial secession schizolytic. Conidia solitary, apical and lateral, simple, ellipsoidal or cylindrical, truncate at the base, pale brown except for the end cells which are subhyaline, smooth-walled, mostly 4-euseptate, rarely 3-euseptate, 26–35 μm long, 7–9 μm thick in the broadest part, 1–1.5 μm wide at the truncate base.

Comments – *Minimelanolocus linderae* is similar in conidial shape to *M. magnoliae* K. Zhang & X.G. Zhang (Zhang et al. 2009), *M. bambusae* (N.D. Sharma) R.F. Castañeda & Heredia, and *M. subulifer* (Corda) R.F. Castañeda & Heredia (Castañeda Ruíz et al. 2001). However, conidia of *M. linderae* are narrower than those of *M. magnoliae* (conidia 10–11 µm wide), and larger than those of *M. bambusae* (conidia 15–19 × 6–7 µm) and *M. subulifer* (conidia 12–29 × 3.5–5.5 µm). Moreover, in *M. linderae* mature conidia are 3–4-euseptate, while those of the three known species are mostly 3-septate. In addition, the mature conidia of *M. linderae* are pale brown, but the cell at each end is subhyaline, which is obviously different from those of *M. magnoliae*, *M. bambusae* and *M. subulifer*.

Minimelanolocus mori Jian Ma & X.G. Zhang, **sp. nov.** Fig. 2

MycoBank MB 561234

Fungus anamorphicus. Coloniae in substrato naturali effusae, brunneae vel atrobrunneae, pilosae. Mycelium partim superficiale, partim immersum in substrato, ex hyphis ramosis, septatis, pallide brunneis vel brunneis, laevibus, 1.5–3 µm crassis compositum. Conidiophora macronematosa, mononematosa, singula, nonramosa, erecta, recta vel flexuosa, septata, laevia, atrobrunnea, apice versus pallidiora, 150–280 µm longa, 3.5–5.5 µm crassa. Cellulae conidiogenae holoblasticae, polyblasticae, in conidiophoris incorporatae, indeterminatae, sympodialiter extendentes, terminales deinde intercalares, brunneae. Loci conidiogeno inconspicuo vel leviter prominentibus, subobscurus. Conidiorum secessio schizolytica. Conidia solitaria, acropleurogena, simplicia, ellipsoidea vel cylindrica, apice rotundata, ad basim truncata, pallide brunnea, cellulis apicali subhyalina vel pallide brunnea, laevia, plerumque 3-euseptata, raro 2-euseptata, 19–25 µm longa, 7.5–10 µm crassa, basi truncata 2.5–3.5 µm lata, cellulis basi 8.5–11 µm longa.

Type: China, Hainan Province: tropical forest of Bairentan, on dead branches of *Morus alba* L. (*Moraceae*), 8 Dec 2010, J. Ma, (**Holotype** HSAUP H5499–1; isotype HMAS 146123).

Etymology: in reference to the host genus, *Morus*.

Anamorphic fungi. Colonies effused on natural substratum, brown to dark brown, hairy. Mycelium partly superficial, partly immersed, composed of branched, septate, pale brown to brown, smooth-walled hyphae, 1.5–3 µm thick. Conidiophores differentiated, single, solitary, unbranched, erect, straight or flexuous, septate, smooth, dark brown, paler toward the apex, 150–280 µm long, 3.5–5.5 µm thick. Conidiogenous cells holoblastic, polyblastic, integrated, indeterminate, sympodially extending, terminal becoming intercalary, brown. Conidiogenous loci inconspicuous or slightly prominent, somewhat obscure. Conidial secession schizolytic. Conidia solitary, apical and lateral, simple, ellipsoidal or cylindrical, apex rounded, base truncate, brown except for the apical cell which is subhyaline or pale brown, smooth-walled, mostly 3-euseptate, rarely 2-euseptate, 19–25 µm long, 7.5–10 µm thick in the broadest part, 2.5–3.5 µm wide at the truncate base, basal cell 8.5–11 µm long.

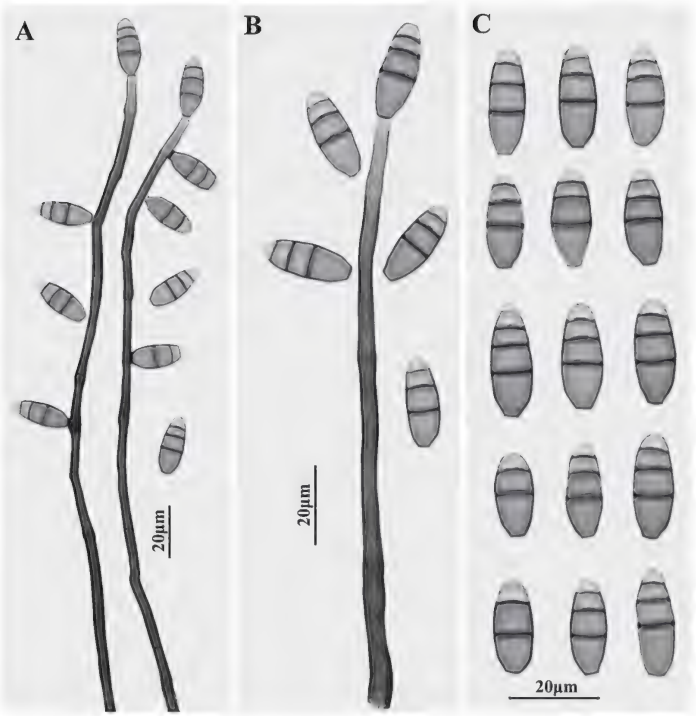

Fig. 2. *Minimelanolocus mori*. A, B. Conidiophores and conidia. C. Conidia.

Comments – The conidia of *Minimelanolocus mori* resemble those of *M. magnoliae*, *M. endospermi* Jian Ma & X.G. Zhang (Ma et al. 2008), and *M. hughesii* (M.B. Ellis) R.F. Castañeda & Heredia (Castañeda Ruíz et al. 2001) in shape. However, *M. mori* differs from *M. magnoliae* (conidia 29–38 × 10–11 μm, basal scar 1–2 μm wide) in its smaller conidia with wider basal scar, and from *M. hughesii* (conidia 12–18 × 4.5–6 μm) in having larger conidia. Moreover, *M. mori* can be separated from *M. endospermi* (conidia 20–30 × 9–12 μm, mainly 2-euseptate) by its slightly smaller mainly 3-euseptate conidia. In addition, the conidia of *M. mori* have a much longer basal cell than is seen in the other three species.

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Two new species of *Passalora* and *Pseudocercospora* from northeastern Uttar Pradesh, India

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Abstract — During surveys of hyphomycetes from northeastern Uttar Pradesh, India, two undescribed taxa were discovered, *Passalora barringtoniigena* sp. nov. on living leaves of *Barringtonia acutangula* (*Lecythidaceae*), and *Pseudocercospora miliusae* sp. nov. on *Miliusa tomentosa* (*Annonaceae*). These two species differ from similar species morphologically. Illustrations and descriptions of the new species are provided.

Key words — biodiversity, foliar diseases, phytopathogenic fungi, taxonomy

Introduction

We have encountered several plant species exhibiting leaf blights during our survey of plant diseases. Critical examination and a thorough survey of the literature revealed that two of these blights were caused by two undescribed species of *Passalora* and *Pseudocercospora. Passalora* is characterised by septate coloured conidia and conidiophores with thickened scars, and *Pseudocercospora* is characterised by septate coloured conidia and conidiophores without thickened scars (Crous & Braun 2003). After comparing the fungi with all other species reported on allied hosts, we found them to be distinct and identified them as new species of *Passalora* and *Pseudocercospora*.

Materials & methods

Infected leaf samples from different parts of northeastern Uttar Pradesh were placed in separate polythene bags and taken to the laboratory. Surface scrapings and free-hand cut sections were prepared from infected portions of the leaf samples and mounted in lactophenol cotton blue on microscope slides. Fungal structures were drawn under 1000× magnification with the help of camera lucida to illustrate all possible details of morphology and ontogeny of reproductive propagules. Measurements were taken with the help of an ocular micrometer. Morphotaxonomic determinations were made with the help of current literature and available resident expertise. Holotypes have been

deposited in HCIO (Herbarium Cryptogamiae Indiae Orientalis), Indian Agricultural Research Institute, New Delhi, India; isotypes were retained in the departmental herbarium for further reference.

Taxonomy

Passalora barringtoniigena R. Singh, Sham. Kumar & Kamal, **sp. nov.** FIG. 1

MycoBank MB 519071

Maculae hypogenae, atro-brunneae vel nigrae. Coloniae hypophyllae, effusae. Mycelium internum. Stromata parva. Conidiophora macronematosa, ex hyphis oriunda singulata vel 2–6-fasciculata, simplicia, erecta vel procumbentia, recta vel flexuosa, geniculata, laevia, crassitunicata, pallide brunnea vel atro-brunnea, 2–7-septata, 22–105 × 3–6 µm. Cellulae conidiogenae integratae, terminales vel intercalares, polyblasticae, cicatricatae, cicatrices incrassatae. Conidia simplicia, sicca, acropleurogena, solitaria, cylindricata, 3–11-septata, recta vel leniter curvata, olivacea vel olivaceo-brunnea, laevia, tenui-tunicata, apices obtusae, ad basim obconicotruncata, hila incrassata, 35–82 × 2–6 µm, hila 1.5–2.5 µm lata.

Type: On living leaves of *Barringtonia acutangula* (L.) Gaertn. (*Lecythidaceae*), Nichlaul Forest, Mahrajganj (U.P.), India, January 2008, coll. Raghvendra Singh, HCIO No. 48783 (**holotype**), GPU Herb. No. KSR-257 (**isotype**).

Etymology: the epithet is derived from the genus name of the host.

Infection spots hypogenous, minute, dark brown to black. Colonies hyphophyllous, effuse. Mycelium internal. Stromata absent or poorly developed. Conidiophores macronematous, arising singly from internal hyphae or in a fascicle of 2–6 from poorly developed stromata, simple, erect to procumbent, straight to flexuous, geniculate, smooth, thick-walled, light to dark brown, 2–7-septate, 22–105 × 3–6 µm. Conidiogenous cells integrated, terminal, becoming intercalary, polyblastic, cicatrized with thickened conidial scars. Conidia simple, dry, acropleurogenous, solitary, cylindrical, 3–11-septate, straight to slightly curved, olivaceous to olivaceous-brown, smooth, thin-walled, apex obtuse, base obconico-truncate, hila thickened, 35–82 µm long and 2–6 µm thick, germinating conidia present, hila 1.5–2.5 µm wide.

Two *Passalora* species previously described on *Barringtonia* are *P. barringtoniicola* (Y.L. Guo) U. Braun & Crous (Crous & Braun 2003) on *B. yunnanensis* from China and *P. barringtoniae-acutangulae* (Kamal et al.) Poonam Srivast. (Srivastava 1994) on *B. acutangula* from India and Australia. *Passalora barringtoniicola* differs from *P. barringtoniigena* in having obclavato-cylindrical, catenate conidia with long curved tips and conidiophores that are densely fasciculate and arise from well-developed subepidermal stromata (Crous & Braun 2003). In *P. barringtoniae-acutangulae* the stromata are well developed, the conidiophores are longer (≤ 240 µm), and the conidia are shorter and wider (17.5–38.6 × 4.2–11.7 µm), 0–3-septate, and obclavate to broadly fusiform (Srivastava 1994).

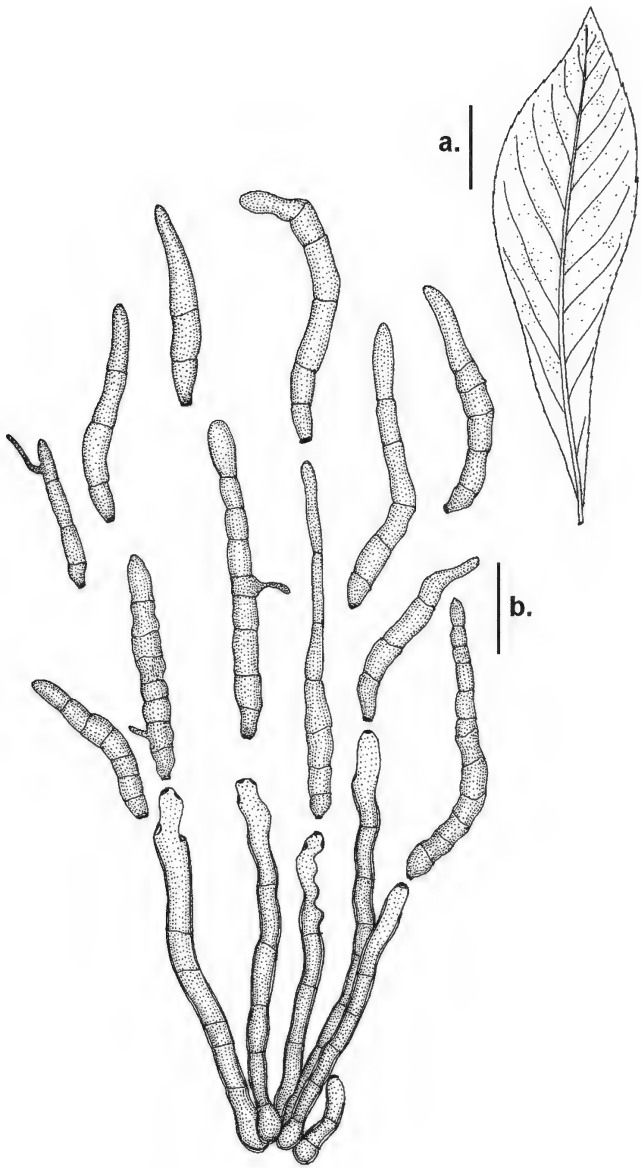

Fig. 1. *Passalora barringtoniigena.*
a: symptoms; b: conidia, germinating conidia, and conidiophores.
(Scale bars: a = 20 mm, b = 20 μm).

Pseudocercospora miliusae R. Singh, Sham. Kumar & Kamal, **sp. nov.** FIG. 2

MycoBank MB 519075

Maculae amphigenae, primo discretae, venulis definitae, demum coalescentes, irregulares et plus minusve, nigrae vel brunneae, 1–21 mm in diam. Coloniae hypophyllae, effusae, brunneae. Mycelium internum. Stromata subepidermalia vel erumpentia, pseudoparenchymatica, 17–21 × 36–42 µm. Conidiophora ex hyphis oriunda singulata vel 2–50-fasciculata, macronematosa, simplicia, cylindricata, erecta vel procumbentia, recta vel curvata, laevia, crassitunicata, ramosa, pallide brunnea, 1–2-septata, 10–70 × 2–5 µm. Cellulae conidiogenae integratae, terminales, monoblasticae, cicatrices non incrassatae. Conidia simplicia, tenui-tunicata, sicca, acropleurogena, recta vel curvata, non ramosa, cylindricata vel obclavata, 0–4-septata, apices subacuta vel acuta, ad basim obconicotruncata vel rotundata, pallide brunnea, 20–58 × 2–3 µm, hila non incrassata, 1–1.5 µm lata.

Type: On living leaves of *Miliusa tomentosa* (Roxb.) J. Sinclair (*Annonaceae*), Vindhyavashini Park, Gorakhpur (U.P.), India, January 2008, coll. Raghvendra Singh, HCIO No. 48786 (**holotype**), GPU Herb. No. KSR-320 (**isotype**).

Etymology: the epithet is derived from the genus name of the host.

Infection spots amphigenous, discrete and vein-limited in the beginning but coalescing to become irregular and more or less necrotic afterwards, black to brown, 1–21 mm in diam. Colonies hypophyllous, effuse, brown. Mycelium internal. Stromata subepidermal to erumpent, pseudoparenchymatous, 17–21 × 36–42 µm. Conidiophores arising singly from internal hyphae or in a fascicle of 2–50 from subepidermal or erumpent stromata, macronematous, simple, erect to procumbent, cylindrical, smooth-walled, thick-walled, branched, light brown, 1–2-septate, 10–70 × 2–5 µm. Conidiogenous cells integrated, terminal, monoblastic, scars unthickened. Conidia simple, thin-walled, dry, acropleurogenous, straight to curved, unbranched, cylindrical to obclavate, 0–4-septate, apex subacute to acute, base obconicotruncate to rounded, light brown, 20–58 × 2–3 µm, hila unthickened, 1–1.5 µm wide.

A thorough survey of the literature indicates that no other *Pseudocercospora* species has been described on *Miliusa tomentosa,* and only one, *Ps. annonacea* (Kamal et al.) U. Braun (Braun 1994), has been described on any *Miliusa* species. In addition to the different host species, *Ps. annonacea* is separated by conidia that are larger (57.5–115 × 3.45–4.6 µm), pluriseptate (up to 15–20-septate) and hyaline. The only other cercosporoid species described on *Miliusa tomentosa, Passalora miliusae* U. Braun & Crous (Crous & Braun 2003), has subhyaline conidia and thickened conidiophore scars.

Pseudocercospora species known to occur the same host family (*Annonaceae*) differ from *Ps. miliusae* as noted below.

In *Ps. aethiopicae* Deighton, conidiophores are shorter (10–40 µm) and always densely fasciculate, never solitary (Deighton 1976).

In *Ps. annonae* U. Braun & Crous conidia (50–150 µm) and conidiophores (20–110 µm) are longer (Braun et al. 2002).

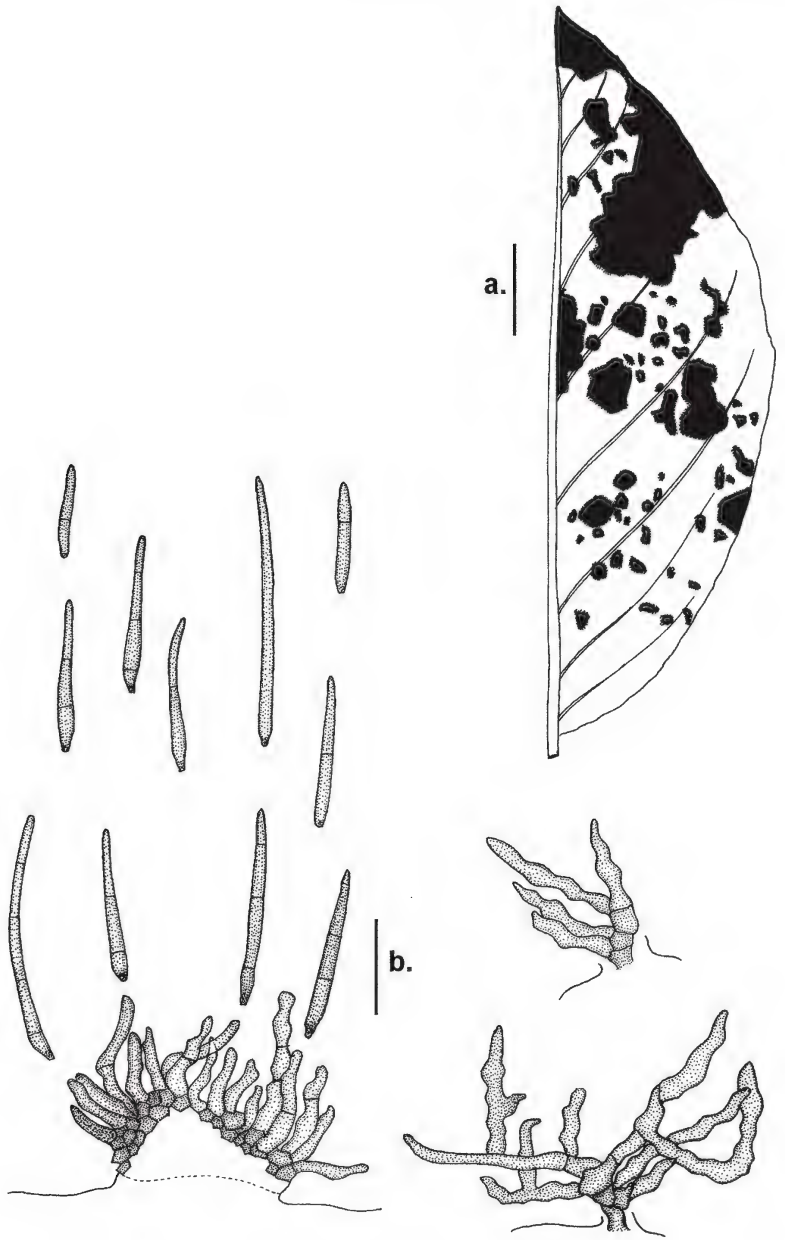

FIG. 2. *Pseudocercospora miliusae.*
a: symptoms; b: conidia, stroma, and simple and branched conidiophores.
(Scale bars: a = 20 mm, b = 20 μm).

In *Ps. annonae-squamosae* U. Braun & R.F. Castañeda, conidia are longer (≤ 75(–85) µm) and 3–7-septate, and the shorter (≤ 30(–45) µm) conidiophores arise singly from superficial hyphae on the lower leaf surface to form large dense (sometimes almost sporodochial) fascicles with well-developed stromata on the upper leaf surface (Braun & Crous 2008).

Pseudocercospora annonarum (Petr. & Cif.) U. Braun & Crous is readily separated by the presence of synnemata (80–250 × 20–50 µm) and conidia that are ellipsoid to ovoid, variably (0–10)-septate, and broader [≤ 8(–9) µm diam.] (Braun & Crous 2008).

In *Ps. annonifolii* (Bat. & Peres) U. Braun & F.O. Freire, the conidiophores are shorter (≤ 20–(30) µm) and conidia longer (≤ 220 µm) (Braun & Freire 2003).

The shorter (6–20 µm) conidiophores of *Ps. asiminae* (Ellis & Morgan) U. Braun & Crous arise from both substomatal and superficial hyphae and the conidia are variably shaped (ellipsoid-ovoid to fusiform), longer (20–80 µm), and with 1–9 transverse (plus occasionally 1–2 longitudinal or oblique) septa and an obtuse apex (Braun & Crous 2008).

The conidia of *Ps. asiminae-pygmaeae* U. Braun are larger (25–140 × 3–6(–7) µm) (Braun & Crous 2008).

Pseudocercospora oblecta (Syd.) Crous & U. Braun possesses sporodochia, shorter (30–50 µm) verruculose conidiophores that taper to a rounded or subtruncate apex of conidiogenous cells that proliferate 1–4 times percurrently, and longer (40–120 µm) verruculose conidia with 1–15 septa and guttules (Braun & Crous 2008).

In *Ps. polyalthiae* J.M. Yen et al. the conidiophores are consistently solitary and arise from superficial hyphae, and the conidia are 3–13-septate and longer (40–156 µm) (Yen et al. 1982).

Pseudocercospora scitula (Syd.) Deighton produces longer conidia (50–110 × 5–8 µm) and conidiophores (60–220 × 4–6 µm) (Deighton 1976).

In *Ps. xenoannonicola* Crous & Bench. both external as well as internal mycelium is present, conidiophores are shorter (10–30 × 3–4 µm), and conidia are longer (30–100 × 2–3 µm) and 5–7-septate (Crous et al. 2000).

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Lecanora wrightiana and *Rhizocarpon inimicum*, rare lichens new to Turkey and the Middle East

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Abstract — During a recent excursion in Iğdır region (Turkey), we sampled lichens from two localities, among which *Lecanora wrightiana* and *Rhizocarpon inimicum* were determined as new to Turkey and the Middle East. Geographic distribution, substrate, chemistry, and comparisons with morphologically similar taxa are presented.

Key words — *Ascomycetes*, biodiversity, parasitic

Introduction

Compared to other countries, not many lichen studies have been conducted in Turkey, so the lichen flora of Turkey remains poorly known. Recently, many lichen taxa have been recorded for Turkey (Yazici et al. 2010a,b,c, Candan & Halici 2009, Candan et al. 2010, Kinalioğlu & Aptroot 2010, Kinalioğlu 2010a,b), but more studies are needed to form a complete lichen flora. No lichenized fungi have previously been reported for Iğdır region.

To date, 27 *Rhizocarpon* species and 95 *Lecanora* taxa have been reported from Turkey. At least 18 parasitic *Rhizocarpon* species are known from Europe (Poelt & Hafellner 1983, Poelt & Vězda 1984, Timdal 1986), but only one parasitic species (*Rhizocarpon epispilum* on *Pertusaria rupicola*) has been found from Turkey (Breuss & John 2004).

Material & methods

Iğdır, one of the poorest forested areas in Turkey, is dominated by steppe. Rich grassy plants cover the surface of the province. Mountain Zordağ (Iğdır: Center) is a well-lit, very high, windswept, open treeless area with gently sloped terrain with streams, grass and rocks. *Prunus*, *Pyrus,* and *Populus* trees are dominant in Üçkaya village (Iğdır: Tuzluca) with *Salix* and *Elaeagnus* occasionally present (Baytop & Denizci 1963). The

climate is characterized by hot and dry summers with moderate precipitation, and cold, snowy winters with high precipitation. Mean annual temperature is 11.6°C. The mean annual rainfall is about 257.6 mm. Mean annual humidity is 63%. (Akman 1999).

The lichen specimens were collected from Iğdır region on 13–14 June 2010. Air-dried samples were examined with Nikon SMZ1500 stereomicroscope and a Nikon Eclipse 80i light microscope. Identifications were determined by consulting keys (Guderley & Lumbsch 1999, Miyawaki 1988, Poelt 1990, Poelt & Vězda 1984, Upreti 1997). Vouchers are stored in the herbarium of the Biology Department, Karadeniz Technical University, Trabzon, Turkey (KTUB).

Species recorded

Lecanora wrightiana Zahlbr., Catalogus Lichenum Univeralis 5: 600. 1928 FIG 1

= *Lecanora japonica* Tuck., Sert. Lich. Trop. Labuan Singapore: 35. 1891, nom. illegit., non Müll. Arg. 1879.

Thallus corticolous, to 1 cm diam., thin, continuous or rimose–areolate to verruculose, slightly yellow-grey, ±cream-white, to slightly greenish–grey, epruinose. Soredia absent. Apothecia sessile to slightly constricted at the base, circular to irregular, 0.2–0.7 mm diam., disc dark red-brown, bright red–brown when wet, epruinose; margin smooth, concolorous with thallus, mostly cream-white, entire. Cortex hyaline, gelatinous, with small crystals. Amphithecium *campestris*-type with small crystals. Parathecium hyaline, without crystals, 15 µm thick. Epihymenium *glabrata*-type, without crystals, red-brown, egranulose. Paraphyses apically ±reticulate and slightly thickened. Asci cylindric-clavate, 12-spored. Ascospores ellipsoid, 13–15 × 7–8 µm. Thallus and margin of apothecium contains atranorin and chloroatranorin. K+ yellow, C–, KC–, P ± yellow.

SPECIMEN EXAMINED: **TURKEY. Iğdır:** TUZLUCA, Üçkaya village, 39°58'13.11"N, 43°39'44.08"E, on *Populus* sp., 1456m, 14.06.2010, KTUB–2018.

REMARKS— By its *campestris*-type amphithecium, *L. wrightiana* is similar to *L. elapheia,* which, however, contains eight spores per ascus and produces triterpenoids.

Lecanora wrightiana occurs mainly on bark and more rarely on rocks in mountain regions. Previously known from Japan and India, it seems to be especially common in Japan (Miyawaki 1988, Guderley & Lumbsch 1999). New to Turkey and the Middle East.

Rhizocarpon inimicum Poelt & Vězda, Herzogia 6(3–4): 471. 1984. FIG 2

Thallus to 3.5 mm in diam., develops its ascomata, disappears at maturity, areolate and parasitic on *Lecanora rupicola*, black-grey, flat at first, soon with massive curved areolae; areolae 0.5–1.5 mm diam., plane at first, later convex; apothecia in small groups, 0.2–0.7(–1) mm; discs dark-brown; proper margin

Fig. 1 (left). *Lecanora wrightiana.* Fig. 2 (right). *Rhizocarpon inimicum.* Scale: 1mm.

black, slightly ± crenulate, curly-raised, ±pruinose, areolate initially, superficial, sessile, immarginate, later moderately marginate in rough, soon concave; hypothecium irregularly, brown; hymenium 120–150 µm high; epihymenium black-brown; hypothecium brown; amphithecium discrete, black-brown, cellular. Asci clavate, ascospores 19–26 × 10–14 µm, ellipsoid, wavering in the middle or constricted, grey-brown to black-brown, 1-septate at first, vertical to crosswise septa later, 4-celled at maturity, photobiont trebouxioid, hypothallus indistinct, pycnidia absent. Thallus K–, C–, KC–, P–. Medulla I ± violet.

Specimens examined: **TURKEY. Iğdır:** Center, Zordağ, 39°45'49.05"N, 43°53'20.13"E, on *Lecanora rupicola*, 2400m, 13.06.2010, KTUB–2020.

Remarks—*Rhizocarpon* species with dark brown or grey-green ascospores placed in subg. *Phaeothallus* —*R. advenulum, R. epispilum, R. inimicum, R. santessonii* and *R. schedomyces*— have 1-septate to submuriform spores (Poelt & Hafellner 1983, Poelt & Vězda 1984), but *R. santessonii* has the smallest ones (Poelt & Vězda 1981). *Rhizocarpon epispilum* does not have an amyloid medulla or vegetative hyphae (Timdal 1986) and produces 2-septate spores, while *R. inimicum* has 1-septate spores with vertical to crosswise septa. *Rhizocarpon advenulum*, *R. epispilum,* and *R. schedomyces* grow on *Pertusaria* spp.; *R. santessonii* grows on *Tremolecia atrata;* and *R. inimicum* grows on *Lecanora rupicola*.

Rhizocarpon inimicum grows parasitically on *Lecanora rupicola* (Poelt and Vězda 1984). Not many parasitic *Rhizocarpon* species have been reported in Turkey, probably because little lichenological research has been performed. If studies on the lichen flora of Turkey are continued, we hope that the number of recorded *Rhizocarpon* species can be increased. Previously known from Spain and Portugal. New to Turkey and Asia.

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We are grateful to Dr. Paolo Giordani and Dr. Javier Etayo for revision of and helpful comments on an earlier draft of this manuscript. This study was supported by TUBITAK (108T566 coded project).

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A new volvate *Macrolepiota* (*Agaricomycetes, Agaricales*) from Italy, with observations on the *M. procera* complex

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Abstract — A new *Macrolepiota* taxon from Italy, *M. rhodosperma* var. *velicopia*, is described and illustrated based on morphological and ITS rDNA data. It is characterized by a well-developed volva and abundant, evident velar remnants on the pileus, a stipe with a minutely squamulose covering, and very thick-walled elements in the pileipellis. A discussion on its taxonomic position within *Macrolepiota* and notes on closely related taxa are provided. DNA sequence analyses support the new taxon within the variability of *M. fuliginosa* sensu Vellinga, a non-volvate taxon that differs from Barla's original sense of *M. fuliginosa.* As Barla did not indicate a holotype in his protologue of *Lepiota procera* var. *fuliginosa* and there are no extant original herbarium specimens, Barla's Fig. 5/PL. 9 (from Les champignons des Alpes maritimes) is selected as a lectotype, and a recent collection from Liguria (Italy) is chosen as the epitype. A recent collection from Sardinia is chosen as epitype for *L. permixta*. Finally, *M. fuliginosa* and *M. permixta* are reduced in rank to forms of *M. procera*, based on their morphology.

Key words — *Agaricaceae,* biodiversity, taxonomy, *Volvolepiota*

Introduction

Macrolepiota Singer is a genus in the *Agaricaceae* characterized by basidiomes with a scaly pileus, a complex annulus, and a stipe with a smooth to granulose-squamulose covering and by spores with a distinct germ pore covered by a hyaline cap (Singer 1986). Species with a distinct volva were accommodated in the genus *Volvolepiota* Singer, proposed in 1959 to replace the illegitimate *Lepiotella* Rick (Rick 1938), a later homonym of *Lepiotella* (E.-J. Gilbert) Konrad.

The type of *Volvolepiota* is the Argentinean species *Lepiotella brunnea* Rick. Singer (1959) added a second species, *V. albida* Singer. In their monograph on *Volvolepiota* Heinemann & de Meijer (1996) argued that the presence of a well developed universal veil in this taxon was not sufficient to warrant a genus separate from *Macrolepiota* but did not propose new combinations in *Macrolepiota*.

Recent ITS-based molecular analyses by Vellinga (2003), Vellinga et al. (2003), and Ge et al. (2010) place *Volvolepiota* species nested in the *Macrolepiota* clade but in a basal position. Vellinga & Yang (2003), who considered *Volvolepiota* a synonym of *Macrolepiota*, proposed new *Macrolepiota* combinations and described *M. velosa,* a new volvate species from China. Ge et al. (2010) proposed *Macrolepiota* sect. *Volvatae* Z.W. Ge et al. to accommodate the volvate species. Four *Macrolepiota* taxa with a volva thus far known (from Australia, East Asia and South America; Vellinga & Yang 2003) all have a tropical distribution and relatively small spores.

Here we discuss our research on an Italian taxon with a copious universal veil and well-developed volva to determine its position within the genus. In the process we also studied *M. fuliginosa*, *M. permixta,* and the complex of *M. procera* (Scop.) Singer.

Materials & methods

Morphology

Macromorphological features were described from fresh specimens. Colour notations in the macroscopic descriptions are from Séguy (1936). Microscopical characters were made from dried material rehydrated in 5% KOH and stained in Congo red, Cresyl Blue, and Melzer's reagent. Spore measurements are based on means of 30 spores from two collections stained in Melzer's reagent. Basidia were measured where widest and from apex (sterigmata excluded) to basal septum. Author citations follow Index Fungorum (http://www.indexfungorum.org/authorsoffungalnames.htm). Herbarium abbreviations follow Thiers (2011). All examined material is housed at TO (Herbarium generale del Dipartimento di Biologia Vegetale, Università degli Studi di Torino, Italy). The new Latin description is deposited in MycoBank (http://www.mycobank.org/).

The following abbreviations are used: Q = quotient of length and width of spores in side view; Q_m = average quotient; L = number of entire lamellae; l = number of lamellulae between each pair of entire lamellae; (Se) = Séguy (1936).

DNA extraction, PCR amplification, and DNA sequencing

Genomic DNA was isolated from 1 mg of herbarium specimens (Table 1) using the DNeasy Plant Mini Kit (Qiagen, Milan Italy) according to the manufacturer's instructions. Universal primers ITS1F/ITS4 (White et al. 1990; Gardes & Bruns 1993) were used to amplify the ITS1+5.8S+ITS2 rDNA region between the small (18S gene) and large (28S gene) subunits. Amplifications were performed in PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) in a 25 µl reaction mixtures containing 5 ng

Table 1. *Macrolepiota* samples newly sequenced in this study.

Sample no.	Species	Country — Location	Collector Sampling date [herb. number]	Habitat	ITS acc. no.
1	*M. fuliginosa*	Italy — Sardinia, Olbia-Tempio, Rena Majore	M. Contu 15/XI/2009 [TO HG2006]	Coastal pinewood	HM246501
2	*M. fuliginosa*	Italy — Liguria, Savona, Borgio Verezzi	A. Vizzini 09/X/1998 [TO AFM11]	*Quercus ilex* litter	HM246502
3	*M. permixta*	Italy — Sardinia, M.S. Vittoria Esterzili	M. Casula 07/XII/2007 [TO AFM12]	*Pinus* sp. litter	HM246503
4	*M. rhodosperma* var. *velicopia*	Italy — Piedmont, Turin, Giaveno, Colletto del Forno	A. Vizzini 07/X/2008 [TO HG2003]	*Fagus sylvatica* litter	HM246505
5	*M. rhodosperma* var. *velicopia*	Italy — Piedmont, Turin, Venaria Reale, Parco Regionale La Mandria	L. Latino 15/X/2008 [TO HG2004]	*Quercus robur* & *Q. petraea* litter	HM246506
6	*M. rhodosperma*	Italy — Piedmont, Turin, Venaria Reale, Parco Regionale La Mandria	A. Vizzini 20/X/2008 [TO HG2005]	*Quercus rubra* & *Corylus avellana* litter	HM246507
7	*M. excoriata* (Schaeff.) Wasser	Italy — Piedmont, Turin, Colle del Frais	A. Vizzini 16/VIII/2008 [TO AFM13]	Pasture	HM246504

DNA, 1x PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 μM of each primer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, and 0.5 unit of Taq polymerase (Promega). PCR run times were 3 min at 95°C (1 cycle), 30 s at 94°C, 45 s at 50°C, 2 min at 72 °C (35 cycles), and 10 min at 72°C for 1 cycle. PCR products were resolved on a 1.0% agarose gel and visualized by staining with ethidium bromide. PCR products were purified with the AMPure XP kit (Beckman) and sequenced by DiNAMYCODE srl (Turin, Italy). Sequences were assembled and edited with the phred/phrap/consed software suite (Gordon et al. 1998). The sequences have been deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

Sequence alignment and phylogenetic analysis

A representative set of the ITS1-5.8S-ITS2 regions of Eurasian and Australian *Macrolepiota* taxa and two *Leucoagaricus* species was retrieved from Genbank. The sequence alignment was performed by MAFFT version 6 (Katoh et al. 2002; Katoh & Toh 2008) using default settings. After minimal visual fine-tuning of the alignment, a final sequence set with a total 775 database length comprised 65 collections from different studies.

A Bayesian Metropolis-coupled Markov chain Monte Carlo (BI) analysis was conducted with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The model of nucleotide substitution for the BI analysis was selected using ModelTest v. 3.7 (Posada & Crandall 1998). The transversional substitution model with gamma correction (TVM + G)

was chosen as the best-fit model based on hierarchical likelihood tests, following the Akaike Information Criterion (AIC). The general structure (lset) of the substitution model was defined using nst=6 rates=gamma ngammacat=4; TVM + G model was gained using the following parameters to define the priors: the four stationary frequencies of the nucleotides (statefreqpr=fixed(0.2310, 0.2236, 0.2349, 0.3105)), the six different nucleotide substitution rates (revmatpr=fixed(1.7916, 5.3304, 2.2627, 0.7366, 5.3304, 1.00)), the proportion of invariable sites (pinvarpr=fixed(0.00)), and the shape (shapepr=fixed(0.3944)). Markov chains were run for 10^6 generations, with the sampling frequency set every 100th generation; all other parameters were used at the default settings. The outgroup *Leucoagaricus nympharum* was selected to root the tree. A burnin value set to 25% of sampled trees was used to calculate the parameters and the consensus tree.

The data set was also analyzed by a maximum likelihood (ML) method using RAxML version 7.2.3 (Stamatakis et al. 2008). One hundred rapid ML bootstraps were performed, using default settings. The phylogenetic trees were visualized and edited with FigTree (http://tree.bio.ed.ac.uk/software/figtree). The alignment was deposited in TreeBase (www.treebase.org) under study accession no. S10573.

Results & discussion

Phylogeny based on the ITS dataset

The ITS amplification products of the analysed samples ranged from 344 to 680 bp. A total of 65 sequences were used for the ITS phylogeny, including the seven newly sequenced collections (Tab. 1) and all others retrieved from GenBank (accession numbers are given in Fig. 3). The multiple sequence alignment was 775 characters long in total.

The phylogenetic trees resulting from the two types of analysis do not differ in topology. The Bayesian inference consensus tree (Fig. 3) indicates that our volvate taxon clusters together with *M. rhodosperma* (P.D. Orton) Migl. (AY243596) and with isolates formerly identified as *M. fuliginosa*, and one accession under the name *M. konradii* (P.D. Orton) M.M. Moser. The two sequences from our volvate *Macrolepiota* differ among themselves and from *M. fuliginosa* and *M. rhodosperma* sequences in only1–2 bases in three different combinations. This clade, supported by a 1.0 posterior probability value and 98% ML bootstrap value, is sister to all other species in sect. *Macrolepiota*, except for *M. clelandii* Grgur.

ITS sequences of taxa identified as *M. permixta* and *M. fuliginosa* do not differ from those of *M. procera*.

The group of *M. mastoidea* (Fr.) Singer, although homogeneous in sequences, represents basidiomes with quite different morphologies, and these have been accessed under a range of species names; an in-depth multigene analysis of this complex is needed.

All three sections, sect. *Macrolepiota*, sect. *Macrosporae* (Singer) Bon, and sect. *Volvatae* are recovered in the phylogenies, and well supported.

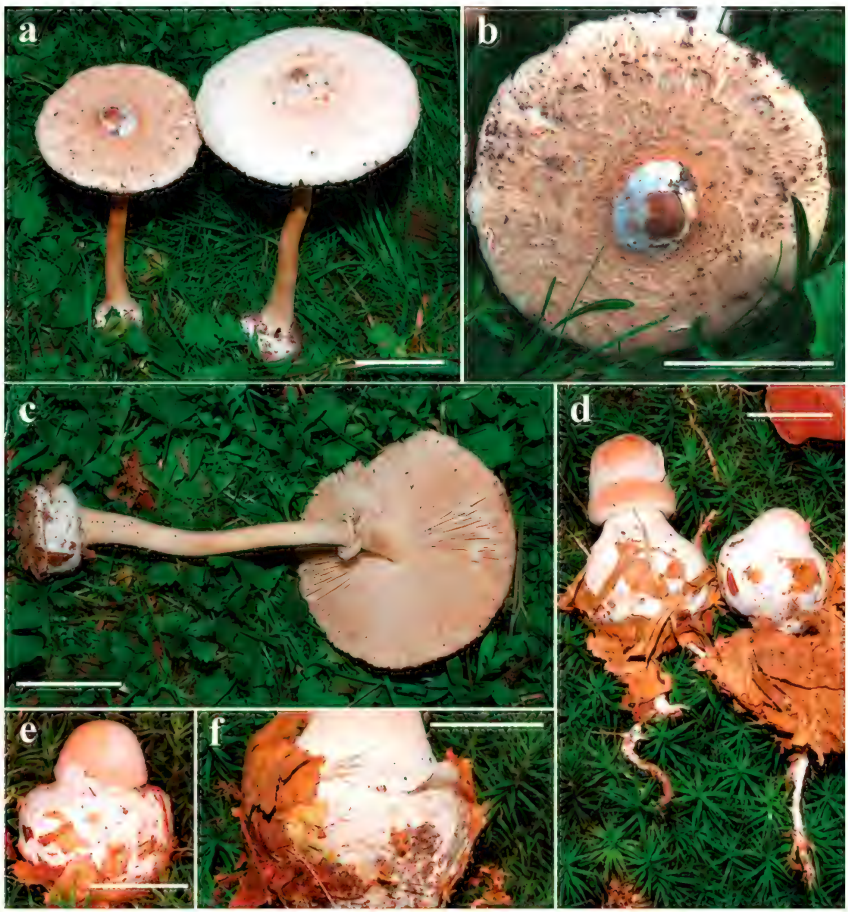

FIGURE 1. *Macrolepiota rhodosperma* var. *velicopia*. Macroscopic features (a–c from the holotype; d–f from TO HG2003). **a**. Mature basidiomes. **b**. Pileus surface with evident velar patches. **c**. Flattened bulb and ornamented stipe. **d**. Young basidiomes covered with universal veil patches and with well developed basal rhizomorphs. **e–f**. Submarginate bulb with free velar limbs (volva). Scale bars: a–c = 5 cm; d–f = 2 cm.

Taxonomy

Macrolepiota rhodosperma var. ***velicopia*** Vizzini & Contu, **var. nov.** FIGS. 1–2

MYCOBANK MB518426

A typo differt velo albo membranoso ad instar Amanitarum in pileo et ad basim stipitis manifesto, hyphis pilei cutis conspicue crassotunicatis, sporis minoribus, fibulisque haud fraequentibus.

HOLOTYPUS — *Italia, in regione Piemonte dicta, ad locum dictum Parco Regionale La Mandria (Venaria Reale, Torino), 15.X.2008, leg. L. Latino (TO HG2004).*

Etymology — the varietal epithet, derived from the Latin words *velum, veli* = veil, velum; and *copiă, copiae* = abundance, emphasizes the copious veil.

Pileus up to 15 cm wide when expanded, at first parabolic then plano-convex, with a low, wide, central umbo, dry, when young entirely covered with a granulose velvety covering, later breaking into a central calotte, which may be round to irregularly star-shaped, felted-subsquamulose, up to ½ of radius wide, cream hazel (Se 134, 695) to light chestnut brown (Se 111, 131), surrounded by 2–10 mm wide and easily removable lighter patches with curled edges, on a radially coarsely floccose-fibrillose covering made up of chamois beige to pale brown fibrillose strands on a white background; with white to dirty white membranous velar remnants as patches on the surface; margin slightly to distinctly fringed and exceeding lamellae. Lamellae, L = 80–140, l = 0–3, free and remote from the stipe, with an evident collarium, moderately crowded, up to 9 mm broad, at first whitish then with cream to pinkish tinges, with a fimbriate-floccose concolorous edge. Stipe protruding into the pileus, (5–) 9–18(–22) × (0.5–)0.8–1.5(–1.8) cm, cylindrical to slightly tapering towards the apex, hollow, bulbous at the base, with an oblong to flattened submarginate bulb, (2–)2.5–3.2(–3.5) × 3–4 cm, often with long white rhizomorphs; with adnate minute cream-hazel zigzagging bands over the whole length, on a white background; bulb white tomentose with evident and membranous white velar remnants on the margin. Annulus movable with age, complex, thickened with a relatively broad edge, upperside creamy and fringed and underside hazel. Context white, unchanging, both on handling and in ammonia vapour, up to 13 mm thick in the pileus; smell indistinct, and taste indistinct to mild. Spore-print whitish-cream.

Spores (13–)13.5–15(–16.5) × 9–10.5(–11.2) µm, on average 14.55 × 9.93 µm, Q = 1.4–1.6, Q_m = 1.46, ellipsoid to oblong, ovoid, hyaline, smooth, thick-walled, with walls easily swelling in 5% KOH, with an apical and central germ-pore (1–1.5 µm wide) and hyaline cap (callus) on it, with a small apiculus, dextrinoid, cyanophilous, congophilous, with inner wall metachromatic in Cresyl Blue (Fig. 2a); basidia 25–42 × 9–16 µm, 4-spored, some 1–2-spored, broadly clavate (Fig. 2b); subhymenium well-differentiated, cellular; hymenophoral trama subregular to slightly interwoven; pleurocystidia absent; lamellar edge sterile, consisting of tightly packed cheilocystidia; cheilocystidia 25–50(–60) × 10–15 µm, abundant, very variable in shape and size, narrowly clavate to sub-fusiform, seldom also lageniform, utriform or cylindrical, sometimes with a small capitulum, rarely with an apical excrescence, often catenulate (pluricellular) septate in lower part, colourless and thin-walled, non-encrusted (Fig. 2c); pileus covering (in calotte) a trichoderm of confusedly erect, (4.5–)6–12 µm wide hyphae ending in a subfusiform (gradually tapering toward the apex) terminal element with several secondary septa (pseudosepta),

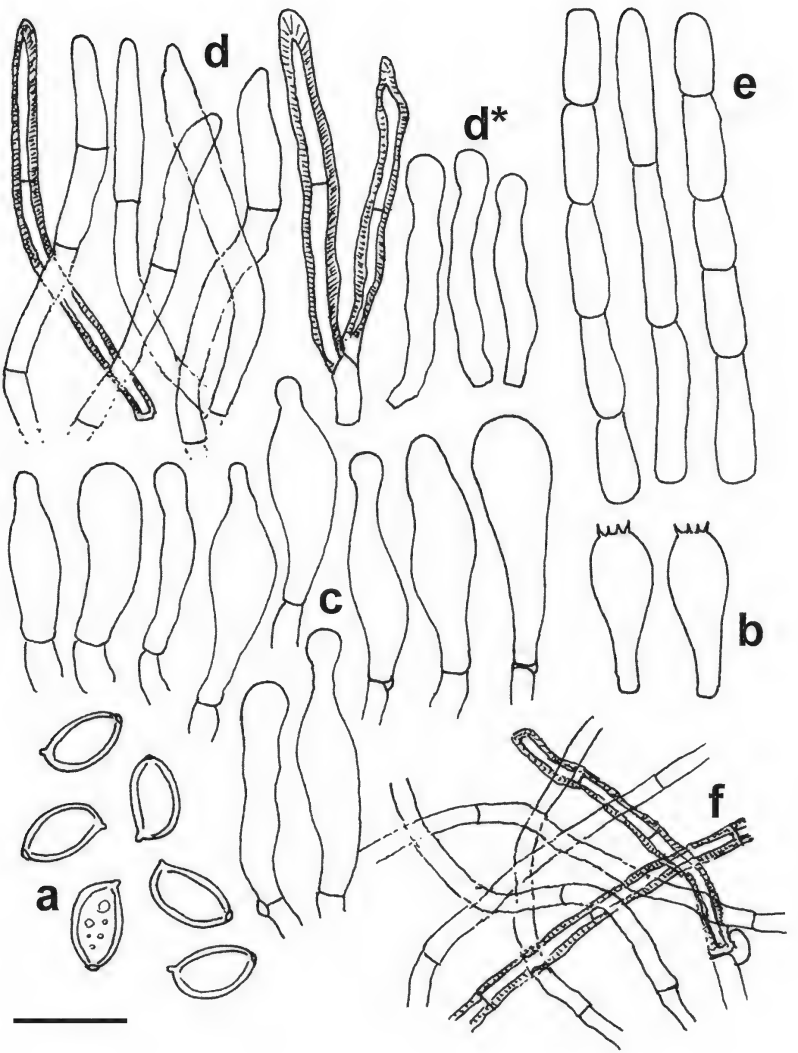

FIGURE 2. *Macrolepiota rhodosperma* var. *velicopia*. Microscopic features (from the holotype). **a**. Spores. **b**. Basidia. **c**. Cheilocystidia. **d**. Pileus covering. **d***. Pileus covering underlying elements. **e**. Annulus elements. **f**. Velar elements. Scale bar: a–c = 20 µm; d–f = 30 µm.

some very thick-walled (1.5–2 µm thick), up to 250 µm long, with a narrow lumen, and then resembling the setiform terminal elements in the pileipellis of *Crinipellis* species, other thin-walled and shorter (Fig. 2d); at the base of the trichodermial elements there is a loose discontinuous layer made up of short cylindrical to subcapitulate elements (Fig. 2d*). Pigmentation in the form of brownish pigment, parietal and cytoplasmatic in the thick-walled hyphae, parietal and minutely encrusting in the thin-walled hyphae, especially towards their base. Annulus consisting of tightly packed, catenulate, articulate, and short cylindroid hyphae, 4.5–9 µm wide, with rare subglobular elements (Fig. 2e). Velar patches made up of elongate, cylindrical hyphae, thin-walled, septate, 3–11 µm wide, sometimes branched or nodulose, with scattered pseudoclamp connections (proliferating clamps), intermixed with thick-walled hyphae (cell wall up to 1.5 µm thick), up to 10.5 µm wide, with internal lumen, looking like the crinipelloid elements present in the pileus covering, probably derived from the underlying pileipellis (Fig. 2f). Clamp connections rare, small, observed at the base of the cheilocystidia; thromboplerous hyphae absent.

Habitat and distribution solitary, terrestrial, on litter of broad-leaved trees, especially *Fagaceae* (*Fagus* and *Quercus*). So far known only from Italy.

Additional material studied: ***Macrolepiota rhodosperma* var. *velicopia*** –**ITALY**, **Piedmont**, Turin, Giaveno, loc. Colletto del Forno, 7 Oct 2008, litter of *Fagus sylvatica* L., 1000 m asl, leg. A. Vizzini (TO HG2003); ***M. rhodosperma* var. *rhodosperma*** – **ITALY**, **Piedmont**, Turin, Venaria Reale, Parco Regionale La Mandria, 20 Oct 2008, litter of *Quercus rubra* L. and *Corylus avellana* L., 230 m asl, leg. A. Vizzini (TO HG2005).

Macrolepiota rhodosperma var. *velicopia* was collected from two different localities in northern Italy. A third collection turned out to be infected by a hyphomycete species that covered the pileus and base of the stipe. Such infections are very rare in the *Agaricaceae*.

Morphologically, *M. rhodosperma* var. *velicopia* is very different from the other volvate *Macrolepiota* species because of such peculiar features as an obviously decorated stipe, spores easily reaching 14–15 µm in length, not strictly cylindrical cheilocystidia, and long very thick-walled elements in the pileus covering. This taxon is the first volvate *Macrolepiota* reported in Europe.

The South American *M. pulchella* de Meijer & Vellinga [≡ *Volvolepiota brunnea* (Rick) Singer] and *M. brunnescens* Vellinga [≡ *V. albida* Singer] differ in smaller spores that rarely reach 11 µm in length, a stipe lacking any decoration, and a less complex annulus; furthermore *M. brunnescens* has a white context turning pink-brown after handling (Heinemann & de Meijer 1996). *Macrolepiota eucharis* Vellinga & Halling from northeast Australia has a smaller pileus (≤ 6 cm broad when fully expanded), a pileus decorated with tiny black scales, a simple ascending annulus that is white on both sides, a uniformly fibrillose dark brown stipe, narrower spores, narrower cylindrical cheilocystidia, and no clamp connections (Vellinga 2003).

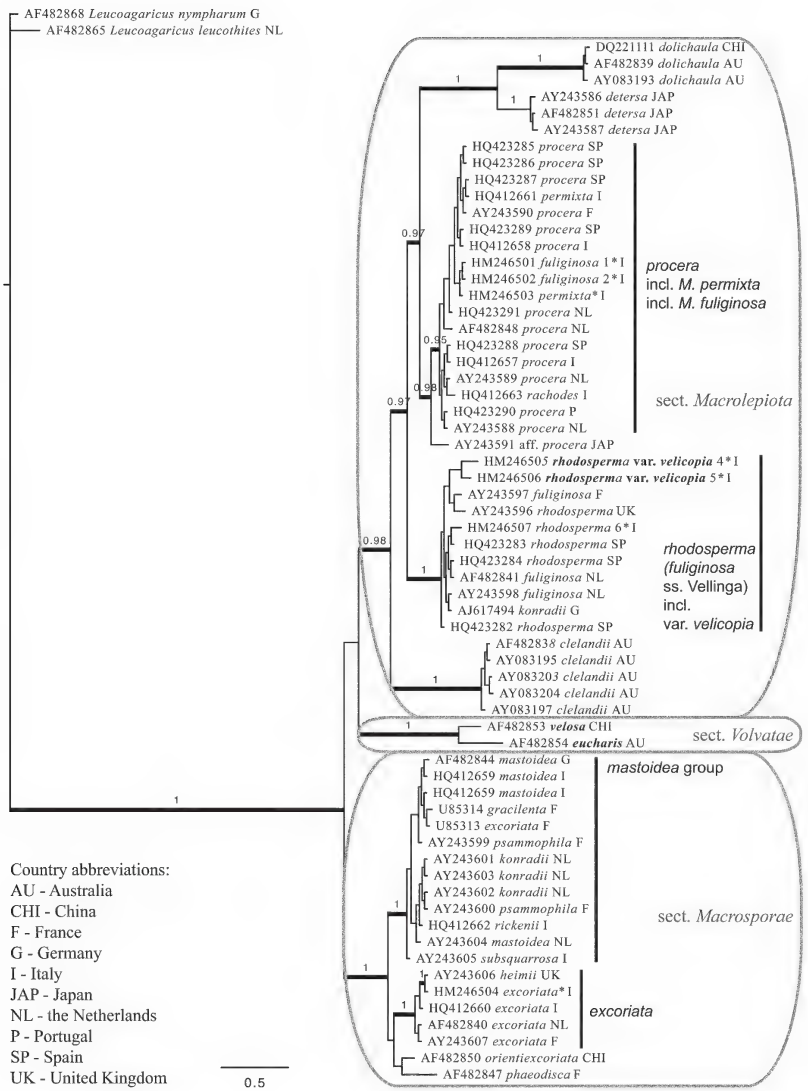

FIGURE 3. ITS-rDNA phylogeny inferred by Bayesian analysis. Bayesian posterior probability values greater than 95%, derived from 10.000 Markov chain Monte Carlo sampled trees, are given above branches; thick branches indicate ≥75% ML bootstrap values from the RAxML analysis. The tree is rooted using *Leucoagaricus nympharum* as outgroup. Volvate *Macrolepiota* taxa are in bold. * indicates samples sequenced in this work and reported in TABLE 1. Names appear as in GenBank except for two recently described species from China (Ge et al. 2010). The bar indicates number of substitutions per site.

Macrolepiota velosa Vellinga & Zhu L. Yang, from south-western China and northern Thailand, is characterized by a 7–9 cm wide pileus with dark brown to purple scales, a finely fibrillose or squamulose stipe, a less complex annulus, very small (8–10(–11) × 6–7 µm) spores, longer and strictly cylindrical cheilocystidia measuring 44–68 × 4.5–7.5 µm, and the absence of clamp connections (Vellinga & Yang 2003; Ge et al. 2010).

Among the poorly studied, insufficiently known taxa, "*M. nordica* var. *subvelata*" Bon ad int. (Bon 1993) comes quite close to our taxon but differs in a less decorated stipe and a different pileipellis structure comprising articulated hyphae.

Our volvate taxon clusters with two *Macrolepiota* taxa that also exhibit thick-walled elements in the pileus covering: *M. fuliginosa* sensu Vellinga (Vellinga 2001, Vellinga et al. 2003) and *M. rhodosperma* lacking such a volva and velar remnants (Fig. 3); *M. rhodosperma* is considered a synonym of *M. fuliginosa* sensu Vellinga (Vellinga 2001, Vellinga et al. 2003).

Our phylogenetic analyses (Fig. 3) clearly demonstrate that *M. rhodosperma* var. *velicopia* is not closely related to the other known volvate taxa (*M. eucharis, M. velosa*), which implies that velar structure acquisition or loss is a homoplastic character that developed independently during the evolution of *Macrolepiota*, which makes it unsuitable for a natural classification of these fungi and further emphasizes the artificial nature of *Volvolepiota*.

Macrolepiota procera* f. *fuliginosa (Barla) Vizzini & Contu, **comb. nov.**

MycoBank MB518708

≡ *Lepiota procera* var. *fuliginosa* Barla, Champ. Alp. Marit.: 21, pl. 9 fig. 5, 1888.

≡ *Leucocoprinus fuliginosus* (Barla) Locq., Bull. Soc. Linn. Lyon 14: 92, 1945.

≡ *Macrolepiota fuliginosa* (Barla) Bon, Docum. Mycol. 7(27–28): 20, 1977.

≡ *Macrolepiota procera* var. *fuliginosa* (Barla) Bellù & Lanzoni, Beitr. Kenntn. Pilze Mitteleurop. 3: 190, 1987.

Type: **FRANCE**, **Alpes-Maritimes**, illustration in Les champignons des Alpes maritimes (Barla 1888: pl. 9 fig. 5, iconotype, **lectotype designated here**). **ITALY**, **Liguria**, Savona, Borgio Verezzi, in *Quercus ilex* litter, 09/10/1998, leg. et det. A. Vizzini (Fig. 5a; TO AFM11, **epitype designated here**).

Lepiota procera var. *fuliginosa*, described from Mediterranean France (Barla 1888), has been differently interpreted by subsequent authors due to the lack of original material (Trimbach 1996). According to mycologists with a vast Mediterranean experience (Bellù & Lanzoni 1987, Candusso & Lanzoni 1990, Bon 1993, Migliozzi 1995), this taxon comes very close to *Macrolepiota procera* from which it differs mainly in the darker tinged pileus and stipe, the hardly disrupted stipe covering, and the browning context. These authors describe the pileipellis of *M. fuliginosa* as a trichoderm made up of thin-walled to only slightly thick-walled hyphae due to the presence of a parietal pigment.

FIGURE 4. *Lepiota procera* var. *fuliginosa* (lectotype, Barla 1888: pl. 9 fig. 5).

Other authors, including Vellinga (2001), Lange & Vellinga (2004), and Lange (2008), described under the name "*Macrolepiota fuliginosa*" a different agaric, characterised in part by a pileus with grey-brown, loose and easily removable scales and a pileipellis comprising very thick-walled elements with brown intracellular and encrusting pigments. This latter taxon is widespread and known from Denmark southwards into Spain (see FIG. 3).

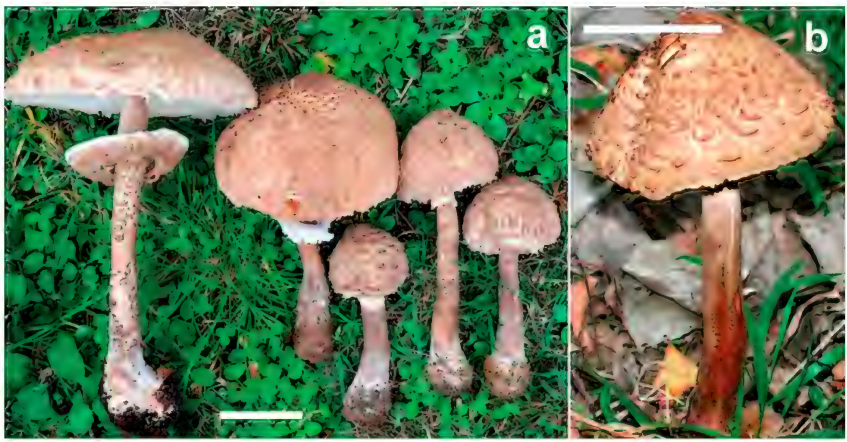

FIGURE 5. **a**. *Macrolepiota procera* f. *fuliginosa* (epitype, TO AFM11).
b. *M. procera* f. *permixta* (epitype, TO AFM12). Bars = 5 cm.

Our ITS sequence analysis, which includes two Mediterranean collections (from Sardinia and Liguria, Italy; TAB. 1), shows that *M. fuliginosa* sensu Vellinga differs from *M. fuliginosa* sensu auct. pl. (FIG. 3). We regard *Lepiota procera* var. *fuliginosa* as a predominantly Mediterranean, xerophilous taxon that is correctly delimited only when based upon Mediterranean records. Consequently, we designate the original Figure 5/Pl. 9 included by Barla (1888) in the protologue (FIG. 4) as its lectotype (iconotype) and select a recent collection from Liguria – where the Mediterranean taxon is rather widespread – as epitype. The basidiomata of the epitypical collection here selected are identical, in gross characters, with those depicted by Barla (1888) and exhibit microscopic features perfectly fitting the descriptions reported by many authors (Bellù & Lanzoni 1987, Candusso & Lanzoni 1990, Bon 1993, Migliozzi 1995) in the region. It is also clear from FIG. 3 that the ITS sequences do not separate this taxon from *M. procera*. However, morphological comparisons do support its recognition as a form of *M. procera* as noted by Bellù & Lanzoni (1987), Candusso & Lanzoni (1990), and Migliozzi (1995). Therefore we introduce the necessary new combination.

The name *Macrolepiota rhodosperma* is available for *M. fuliginosa* sensu Vellinga. Notes on the synonymy of the two are given by Vellinga (2001) and Vellinga et al. (2003). A good picture is provided by Breitenbach & Kränzlin (1995, fig. 250 as *M. konradii)*. *Macrolepiota rhodosperma* var. *rhodosperma,* which lacks the copious velar remnants of var. *velicopia*, also has longer spores and a context that changes brown-red.

FIGURE 6. *Lepiota permixta* (Barla 1888: pl. 11 figs 1–4).

Macrolepiota procera f. ***permixta*** (Barla) Vizzini & Contu, **comb. nov.**

MYCOBANK MB518709

≡ *Lepiota permixta* Barla, Bull. Soc. Myc. Fr. 2: 114, 1886.

≡ *Leucocoprinus permixtus* (Barla) Locq., Bull. Soc. Linn. Lyon 14: 92, 1945.

≡ *Macrolepiota permixta* (Barla) Pacioni, Micol. Ital. 8(3):13, 1979.

≡ *Macrolepiota procera* var. *permixta* (Barla) Quadr., in Quadraccia & Lunghini, Quad. Acc. Naz. Lincei 264: 110, 1990.

Type: **FRANCE**, **Alpes-Maritimes**, Bendejeun, 7/12/1893, Barla Myc.283 (NICE, **lectotype**, designated in Trimbach 1996: 226). **ITALY**, **Sardinia**, M.S. Vittoria Esterzili, in *Pinus* litter, 07/12/2007, leg. M. Casula, det. M. Contu (Fig. 5b; TO AFM12, **epitype designated here**).

Barla (1886) described *Lepiota permixta* as a large long-stemmed species characterised by a pileus that is "10–15" cm wide, "brun-cannelle-fauve au centre," "brunâtre" towards the margin, "à écailles plus ou moins apprimées, fibrilleux, blanchâtre et plus ou moins écorché vers la marge", lamellae that are yellowish-white or "charné-clair", a very slender white stipe with "petites écailles appliquées, irrégulières, brunâtres, écorché vers la marge", a membranous "fauve-brunâtre" annulus, and a reddening context. He added that the described basidiomes were collected in a submontane region and that the species was rare. In the short notes accompanying the French description, Barla stated that *Lepiota permixta* combined features from three species: the slender habit of *L. procera*, the "écorché à la marge" pileus of *L. excoriata*, and the reddening context of *L. rachodes*. The colour plate later published by Barla (1888) displays basidiomes exhibiting the very same features as described in the protologue and with an annulus that apparently differs from that typically described for *Macrolepiota procera* complex (Fig. 6). Most mycologists, especially those in the Mediterranean region, have treated *Lepiota permixta* as a species separate from the *M. procera* complex, as supported by the clearly reddening context (e.g., Pacioni 1979 with a very good description of Sardinian material, Bon 1993, Bellù & Lanzoni 1987, Pázmány, 1985). Others (Candusso & Lanzoni 1990, Migliozzi 1995, 1997, Quadraccia & Lunghini 1990) consider *M. permixta* a variety of *M. procera*, assuming that the presence or absence of reddening is not sufficient to distinguish the two taxa at species level. Pázmány (1988) showed the great macromorphological variability of *M. procera* by describing eight infraspecific taxa. Since our selected *L. permixta* epitype and other collections identified as *M. permixta* share nearly or completely identical ITS sequences with *M. procera* and *M. fuliginosa*, we reduce *M. permixta* to a morphological form of *M. procera* and propose a new taxonomic combination.

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Studies in *Amanita* (*Amanitaceae*) of Central America. 1. Three new species from Costa Rica and Honduras

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ABSTRACT—*Amanita conara*, *A. costaricensis*, and *A. garabitoana* are proposed as new species. These taxa are added to twelve previously described species known from, or reported here for the first time from, the region of study: *A. advena*, *A. arocheae*, *A. brunneolocularis*, *A. colombiana*, *A. eburnea*, *A. farinosa*, *A. flavoconia* var. *inquinata*, *A. fuligineodisca*, *A. muscaria* subsp. *flavivolvata*, *A. polypyramis*, *A. sororcula*, and *A. xylinivolva*. *Amanita flavoconia* var. *sinapicolor* is proposed to be a taxonomic synonym of *A. flavoconia* var. *inquinata*. An unusual species of *Amanita* subsection *Vittadiniae* is given the code *Amanita* sp. HON1 and treated only in a key to regional species of *Amanita* section *Lepidella*. A gazetteer is provided for Costa Rican sites at which *Amanita* species have been collected.

KEY WORDS—Área de Conservación Guanacaste, Cordillera Talamanca, Mexico, Andean Colombia, taxonomy

Introduction and overview

This paper addresses the genus *Amanita* Pers. as a part of the extensive study of macromycetes undertaken by Halling, Mueller, and numerous colleagues in Costa Rica.

The history of work investigating the diversity of Costa Rican macrofungi [see discussions in (Halling & Franco-M. 1996; Halling & Mueller 1999, 2005; Baroni and Halling 2000; Ammirati et al. 2007)] has been long, but intermittent. Currently there is an emphasis on obtaining additional data on the fungi of Costa Rica through the Costa Rican National Biodiversity Inven-

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Fig. 1. *Amanita garabitoana*. a. Habit (Halling 8198) (×0.25±). b. Habit (holotype) (×0.27). Photos by R.E. Halling (a) and R.E. Tulloss (b).

tory—a multinational project coordinated by the Costa Rican National Biodiversity Institute (INBio) (Mueller & Mata 2001). Work to date has documented a highly diverse macrofungal community (Mueller & Halling 1995; Carranza & Mueller 1996; Halling & Mueller 1997, 2002; Mueller et al. 2006). To date our investigation of Costa Rican *Amanitaceae* indicates there are at least 35 species of *Amanita* in the country; of these, a number will be new to science.

Materials and Methods

Methods and terminology follow those of Tulloss et al. (1992) and expansions by Tulloss (1993, 1994, 1998b, 2000a). A recent terminological summary appeared in (Tulloss & Lindgren 2005) and is available on-line (Tulloss 2011a).

Samples from selected exsiccata revised during research reported herein have been supplied to three laboratories now carrying out phylogenetic studies related to *Amanita* sections *Caesareae* and *Lepidella*.

Color codes of the form "6D6" are from (Kornerup & Wanscher 1978). Color codes of the form "10YR 4/3" are from the Munsell soil color charts (Anonymous 1975). Color codes of the form created by the Inter-Society Color Council and the U.S. National Bureau of Standards were translated to Munsell notation using (Kelly 1965; McKnight 1977). Color names with initial capital letters (e.g., Empire Yellow) are from Ridgway (1912), and conversions from Ridgway's names to Munsell color codes is based on the work of Hamly (1949).

The form of author citations follows Kirk & Ansell (1992, 2010).

"P. N." stands for "Parque Nacional." The nonitalicized abbreviation "std." stands for "standard deviation." With two exceptions ("HKAS"—Kunming Institute of Botany, Academia Sinica, China; and "RET"—Tulloss' personal herbarium), codes for herbaria follow Index Herbariorum (Holmgren et al. 1990; Thiers 2010).

In the lists of collections examined, locality names in Costa Rica are provided in abbreviated form. Known site data for these abbreviated names are provided in the appendix to this paper.

Taxonomic part

Extensive collecting in Costa Rica in the last fifteen years has much improved understanding of the genus *Amanita* in that country. We estimate the number of species in the genus present in Costa Rica alone to be 35 to 40. To date, 12 previously described taxa of *Amanita* have been collected and determined or described as new from Costa Rica and Honduras. Previously described taxa are provided in the following list—organized by section of the genus. When range data in a list entry includes Colombia, the source is Tulloss et al. (1992).

Amanita sect. *Amanita* (three taxa):

- *A. farinosa* Schwein. – Costa Rica is the known southern limit of distribution. It was described from North Carolina, USA; the range of this mushroom extends northward into Prov. Québec, Canada (Pomerleau 1980).
- *A. muscaria* subsp. *flavivolvata* Singer – originally described from *ca.* San Francisco, California, USA, this species now is known to have a range extending north into the Alaskan panhandle and eastward across Canada and the northern United States to Newfoundland in the north and extending southward at least to Costa Rica. In the southeastern U.S. its distribution is unclear due to confusion with *A. muscaria* var. *persicina* Dav. T. Jenkins (Jenkins 1977, 1986), which is endemic in that region. In North America, a yellow-capped color-variant of subsp. *flavivolvata* dominates in most of that part of the region of distribution north and east of the Great Plains. The typical, red-capped color-variant dominates throughout the remainder of the range. In the neovolcanic region of Mexico, the subspecies is relatively common in mixed montane forest; and is associated with *Quercus* in Costa Rica. If not native in Colombia, it has been introduced there with *Pinus* or *Quercus* from more northerly latitudes. The taxon is often misreported throughout its range as the type subspecies or type variety of *A. muscaria* (L. : Fr.) Lam.(Tulloss 2009c; Geml et al. 2008).

- *A. xylinivolva* Tulloss, Ovrebo & Halling – This species was described from Andean Colombia. Cochise Co., Arizona, is the known northern distribution limit (Tulloss 2011b).

Amanita sect. *Caesareae* Singer: no previously described taxa known.

Amanita sect. *Vaginatae* sensu Zhu L. Yang (Yang 1997) (three taxa):

- *A. colombiana* Tulloss, Ovrebo & Halling – Costa Rica is the known northern limit of distribution. The southern limit is in Andean Colombia.
- *A. fuligineodisca* Tulloss, Ovrebo & Halling – Honduras is the known northern limit of distribution. The southern limit is in Andean Colombia.
- *A. sororcula* Tulloss, Ovrebo & Halling – Costa Rica is the known northern limit of distribution. The southern limit is in Andean Colombia.

Amanita sect. *Lepidella* sensu Bas (1969) (two taxa):

- *A. advena* Tulloss, Ovrebo & Halling – Costa Rica is the known northern limit of distribution. The southern limit is in Andean Colombia. Previous to our work in Costa Rica, the only known collection of this species was the Colombian holotype.
- *A. polypyramis* (Berk. & M. A. Curtis) Sacc. – Costa Rica is the southern limit of known distribution. The known northern limit is in the sandy Atlantic coastal plane of Cape Cod, Massachusetts (Tulloss 2009b). The species was described from North Carolina.

Amanita sect. *Amidella* (E.-J. Gilbert) Konrad & Maubl.: no previously described taxa known.

Amanita sect. *Phalloideae* (Fr.) Quél. (two taxa):

- *A. arocheae* Tulloss, Ovrebo & Halling – No change in known range (central Mexico to Andean Colombia).
- *A. eburnea* Tulloss (Tulloss 1989) – No change in known range (Belize and Honduras).

Amanita sect. *Validae* (Fr.) Quél. (two taxa):

- *A. brunneolocularis* Tulloss, Ovrebo & Halling – This species was originally described from Costa Rica and Colombia. It has now been found under *Pinus* in North Carolina, USA, along a well-traveled highway (Tulloss 2009d).
- *A. flavoconia* var. *inquinata* Tulloss, Ovrebo & Halling – The known northern limit of the range of this taxon is in the neovolcanic zone of central Mexico. Field observation in Costa Rica has led us to conclude that *A. flavoconia* var. *sinapicolor* Tulloss, Ovrebo & Halling comprises material of var. *inquinata* that has been exposed to direct sun-

light at higher altitudes resulting in some desiccation and alteration of pigment. As has been observed in other taxa of *Amanita* (e.g., Tulloss & Borgen 1996; Tanghe & Hillhouse 1973), the desiccation also results in production of smaller and more nearly globose spores. As previously observed, (Tulloss et al. 1992), there is a strong statistical correlation between cap color and spore shape; but we now believe this was wrongly interpreted as genetically determined by the authors of the pale variety. Therefore, we propose reducing *A. flavoconia* var. *sinapicolor* to synonymy with var. *inquinata*.

1. Section *Caesareae*

At present we believe that there are as many as 3 taxa of *Amanita* sect. *Caesareae* in Costa Rica (Tulloss 2009e). These include no taxa known previously from Andean Colombia (Tulloss et al. 1992). In this paper we propose a new species easily distinguishable in the field, in *Amanita* stirps *Hemibapha* (Tulloss 1998a, Tulloss 2009a)—*A. garabitoana*.

Amanita garabitoana Tulloss, Halling & G.M. Muell. **sp. nov.** FIGS. 1–4
MYCOBANK MB 518295

Fig. 2. *Amanita garabitoana*. Habit (Tulloss 6-16-95-D), (a. ×0.38. b. ×0.31.). Photos by R.E. Tulloss.

Pileus 60–212 *mm latus, aurantiobrunneus vel olivaceoflavus vel flavobrunnea vel aurantioflava, disco olivaceobrunneo vel aurantiobrunneo vel rubrobrunneo, saepe zona brunnea circa ad extremum intimum striae marginalium, margine longistriato, nonappendiculato. Lamellae subadnatae vel liberae, confertae, subroseocremeae vel subaurantiocremeae vel pallidocremeae vel subalbae vel albae; lamellulae subtruncatae vel truncatae. Stipes* 95–258 × 15–24 *mm, subaurantioflavus vel pallidobubalinus vel subcremeus, bulbo nullo, annulatus, frustris coactis flavis vel sordidoflavis vel olivaceoflavis vel brunneis vel aurantiis partis superae limbi interni veli universalis ornatus; velum universali saccatum, robustum, membranosum,* 48–96 × 30–45 *mm, pagina externa alba, pagina interiori subaurantia vel pallidobrunnea. Fibulae praevulgaris. Basidiosporae* (7.5–) 8.0–11.0 (–13.6) × (5.7–) 6.5–8.4 (–9.9) *µm,* (**L** = (8.3–) 8.7–10.0 (–10.3) *µm;* **L'** = 9.4 *µm;* **W** = (6.6–) 6.9–7.7 (–7.8) *µm;* **W'** = 7.3 *µm;* Q = (1.06–) 1.15–1.43 (–1.73); **Q** = (1.22–) 1.24–1.37 (–1.40); **Q'** = 1.29), *nonamyloideae, late ellipsoideae vel ellipsoideae. In quercetis tropicalibus montanis costaricensibus et hondurensibus habitat. Amanita arkansana Amanitae stirpis Hemibaphae simulanissima. Species nova ob distributionem geographicam, pileum et stipem relative robustiores, ordinationem colores, colores variabiles ob aetatem vel contusa, et magnitudinem sporarum.*

Holotype — Costa Rica, Prov. San José, San Gerardo de Dota no. 1, Tulloss 6-21-95-G (USJ).

Etymology — In honor of Garabito, an indigenous military-political leader of the Huetares, a people of the Central Valley of Costa Rica. Garabito resisted Spanish occupation of Costa Rica until his capture in 1574. He fought to maintain the cultural heritage of his people and to oppose both the mistreatment of indigenous peoples by the Spanish and the establishment of Catholicism.

Illustrations: Rossman, et al. (1998: cover [Tulloss 6-16-95-D]); Tulloss (2000: 16 (fig. on right) [Tulloss 6-16-95-D]); Halling & Mueller (2005: 32 [Halling 8138], 173 [Halling 8438]).

Pileus: 60–212 mm wide, orange-brown (e.g., 5C6-7) to olivaceous yellow or yellowish brown (4B-C7) to olivaceous tan (more orange than 4B8) to orangish yellow (4A6), darker over disc [e.g., olive brown (more olivaceous than 5D7-8) to orange-brown (6C8) to red-brown (6E6-7, 10F5)], often with dark zone (brown to chestnut brown) at inner end of marginal striations, drying dark brown, campanulate to strongly convex to convex to planoconvex with slightly depressed disc, with large broadly subconic umbo, viscid to tacky to dry (then subshiny), often dull, subglabrous to glabrous, silky fibrillose to fibrillose to somewhat finely pruinose over disc; *context* white to pale sordid white to pale yellowish white outside of disc, yellow (3A3) to pale yellow under pileipellis and above stipe in disc or above lamellae, 4.5–12.5 mm thick at stipe, thinning evenly for 75–85% of radius, then membranous to margin; *margin* strongly striate to plicate-striate [(0.15R–) 0.5R–0.75R], incurved at first, remaining at least somewhat decurved, rounded serrate to eroded, nonappendiculate; *universal veil* absent.

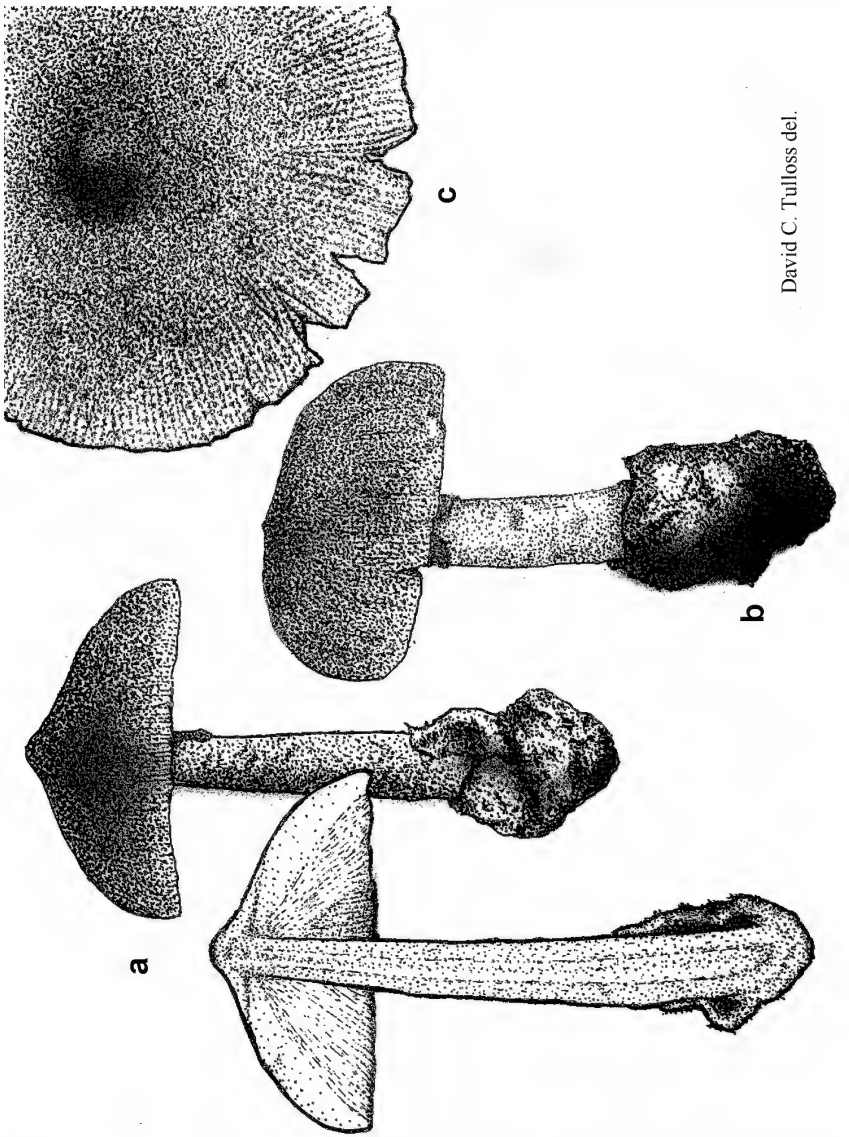

Fig. 3. *Amanita garabitoana* habit. a. Tulloss 6-16-95-D (×0.33). b. Tulloss 6-15-95-H p.p. (×0.57). c. Tulloss 6-15-95-H p.p. (×0.39).

LAMELLAE: adnate to narrowly adnate to free, sometimes with short decurrent line (rarely extending to partial veil, sometimes requiring 10× lens) on stipe,

close to crowded, faintly pinkish cream to pale orangish cream to pale yellowish cream to light cream to pale yellow to dull white to off-white in mass, white to off-white to pale yellowish white in side view, 4.5–23 mm broad, broadest at about 75% of radius from stipe, thin to moderately thick, with entire and concolorous edge, some forked near margin, with occasional reverse forking; *lamellulae* rounded truncate to subtruncate to truncate (less frequently subattenuate to attenuate, but commonly so in one collection), unevenly distributed, of diverse lengths, plentiful.

STIPE: 95–258 × 15–24 mm, with ground color light orangish yellow (ca. 4A5) to pale yellow (3A3) to buff to yellowish white to off-white, yellow and pruinose at apex, unchanging or with ground taking on more orange tint or becoming more sordid with age, cylindric or narrowing upward, barely or not at all flaring at apex, below partial veil decorated with dry ochraceous to dull brownish orange to light orange-brown (5C5) to yellow brown (ca. 4B7-8) to yellow (2A3-4) floccose to fibrillose scales (becoming darker or more orange from handling), below annulus minutely fibrillose or finely striatulate (especially with age); *context* white to pale yellow (2A2) to pale yellow-orange (4A3), unchanging, with larval tunnels concolorous, stuffed with white glistening fibrillose material moderately loosely packed, becoming hollow, with central cylinder 3–10 mm wide; *partial veil* with upper surface light yellow to slightly greenish yellow (3A4) to dull yellow (4A4-5) to sordid yellow (more sordid than 4A8, more sordid than 4B4) to moderate yellow (not as green as 3A4), subsuperior to subapical to apical (e.g., attached for 5± mm, with 3–15 mm free), membranous, thin, copious, skirt-like, persistent, eventually collapsing on stipe and becoming sordid yellow, striate on upper surface, smooth below; *universal veil* as saccate volva almost always connected at (or very near to) base of stipe, often with considerable rather firm portion below stipe base, 48–96 × 30–45 mm (often entirely below substrate surface), membranous, soft, with outer surface white (sometimes with orange-brown discoloration) and inner surface pale olivaceous tan to pale orangish white [may become browner (paler than 10YR 8/6) with age], with context white, 1.5–4± mm thick, with limbus internus not always distinct or well preserved [when present, largely white or largely concolorous with material decorating stipe, at variable distance from point of attachment of volva and stipe, at first firmly connected to sordid yellowish felted material (with latter eventually becoming stipe-decorating squamules and patches)].

Odor mild, indistinct, or fungoid. *Taste* indistinct.

MACROCHEMICAL TESTS: Laccase test (syringaldazine): in material just maturing, negative throughout basidiome. Tyrosinase test (paracresol): in material

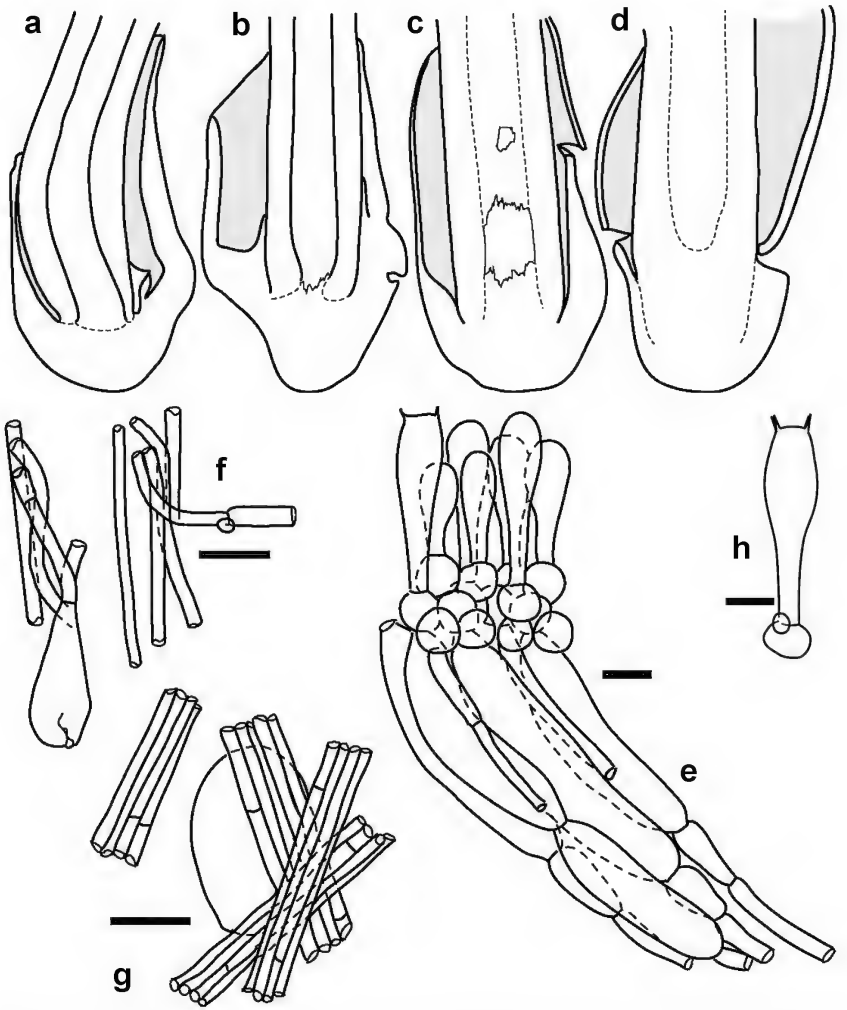

Fig. 4. *Amanita garabitoana*. a-d.Variation of stipe base attachment to universal veil and variation of limbus internus (a. Tulloss 6-16-95-C. b. Tulloss 6-16-95-K. c. holotype. d. Tulloss 6-15-95-H). e. Elements of hymenium and subhymenial tree (isotype). f. Elements of partial veil interior (Schmit 475). g. Elements of partial veil viewed from underside (isotype). h. Basidium with clamp (Tulloss 6-16-95-C). Scale bars = 10 µm.

just maturing, positive in cap context, pileipellis, lower half of stipe context, universal veil except for very base of volva, partial veil, and on edges (including breaks and cuts) of lamellae. Test voucher: Tulloss 6-21-95-G.

PILEIPELLIS: 75–100 µm thick; subpellis yellow-orange, 65–90 µm thick; suprapellis minimal, pallidly concolorous to colorless, extensively gelatinized, 10± µm thick, intimately connected to collapsed hyphal detritus of universal veil (see below); filamentous undifferentiated hyphae 1.0–5.0 µm wide, branching, dominating, subradially oriented, densely packed vertically, sometimes with hyphal tips expanded slightly at apex; vascular hyphae 1.4–7.1 µm wide, common, sinuous, infrequently branching, without dominant orientation. PILEUS CONTEXT: filamentous undifferentiated hyphae 0.9–8.5 µm wide, plentiful, branching, frequently fasciculate, forming matrix loosely to densely interwoven around acrophysalides; acrophysalides of two forms, away from stipe apex plentiful narrowly clavate to elongate (e.g., 152 × 28 µm, 95 × 42 µm), above stipe apex dominating and densely packed with longitudinal orientation like stipe context acrophysalides and ovoid to elongate ovoid to subpyriform (up to 74 × 46 µm or larger), in umbo greatly reduced in number; vascular hyphae 2.5–17.8 µm wide, branching (especially frequently in region above stipe in disc), sinuous to looping and interweaving in loose "knots," common locally, especially common near pileipellis and in region above stipe in disc; clamps plentiful. LAMELLA TRAMA: bilateral, divergent; w_{cs} = 25–45 µm; subhymenial base dominated by curved intercalary inflated cells (up to 94 × 22 µm) sometimes as concatenated pair (together up to 111 µm long) and then arising from filamentous undifferentiated hyphae arising in central stratum, otherwise arising from short partially inflated clavate segment in manner of chained pair, giving rise to cells of subhymenium; central stratum containing intercalary narrowly fusiform cells (e.g., 62 × 14.0 µm); filamentous undifferentiated hyphae 3.2–4.9 µm wide, with those in subhymenial base sometimes giving rise to cells of subhymenium; vascular hyphae 2.0–11.3 µm wide, occasionally branching, often sinuous, uncommon to locally common; clamps common in central stratum. SUBHYMENIUM: w_{st}-near = 105–130 µm (crushed?); w_{st}-near = 95–105 (very good rehydration, estimated from measurement and partially schematic drawing); w_{st}-far = 150 µm (crushed?); w_{st}-far = 100–115 µm (very good rehydration, estimated from measured data and schematic drawing); cellular (pseudoparenchymatous) or dominated by inflated cells, with cells in 2 to 3 layers (1 to 2 layers below longest basidia), with some uninflated branched or unbranched elements arising from inflated cells of subhymenium and giving rise in turn to basidia/-oles, otherwise with basidia arising from inflated cells up to 12.0 × 10.0 µm. BASIDIA: 29–56 (–59) × (8.1–) 8.5–13.0 µm, dominantly 4-sterigmate, rarely 5-sterigmate in immature material, with sterigmata up to 5.5 × 2.0 µm; clamps and proliferated clamps plentiful, prominent. UNIVERSAL VEIL: *On pileus*: at least in moist conditions persisting to maturity in thin layer (25– 45 µm thick) and sometimes

giving hoary appearance to pileus, at 1250× in cross-section giving appearance of somewhat sparse "curly hair"; filamentous undifferentiated hyphae dominant, collapsed, partially gelatinized, mostly under 2.0 µm wide, curling or coiling, branching, without dominant horizontal orientation; inflated cells very infrequent, soon collapsed and gelatinized; vascular hyphae without dominant orientation, of width similar to other hyphae, moderately frequent, scattered. *On stipe base, exterior surface*: partially gelatinized, in a relatively shallow layer, interior visible through occasional gaps; filamentous undifferentiated hyphae 1.7–4.2 µm wide, in fascicles up to 12 or more hyphae wide or singly, densely criss-crossed and interwoven, with many larger fascicles longitudinally oriented, with occasional openings giving (on whole) appearance of openings in expanded net shopping bag. *On stipe base, interior*: rather dense lattice-like structure of plentiful interwoven filamentous undifferentiated hyphae, enclosing globose to ellipsoid to clavate cells (up to 66 × 38 µm), with such cells plentiful to locally dominating in regions somewhat distant from exterior surface layer, enclosing smaller less frequent often clavate cells near exterior surface layer. *On stipe base, inner surface*: like interior but broken and gelatinized, indicating gelatinization takes place within universal veil or at interface between universal veil and pileipellis. STIPE CONTEXT: longitudinally acrophysalidic; filamentous undifferentiated hyphae 2.0–7.0 µm wide, branching, rather common away from surface, becoming dominant and strongly longitudinally oriented toward outer surface, at which forming dense pellis-like layer; acrophysalides up to 182 × 24 µm, smaller near exterior surface, with walls thin or up to 0.7 µm thick, with some near surface having yellowish walls; vascular hyphae 2.1–10.1 µm wide, sometimes sinuous, infrequent, unevenly distributed. PARTIAL VEIL: underside strongly gelatinized; upper side bearing plentiful remnants of inflated cells from former interface to lamellae edges; filamentous undifferentiated hyphae 1.5–4.5 µm wide, branching, dominant in interior, dominantly subradially oriented, dominantly fasciculate (with fascicles mostly 4 to 5 hyphae wide); inflated cells of interior common, clavate, to elongate ellipsoid, thin-walled, up to 28 × 17.5 µm, terminal, solitary, often with subradial orientation of longer axis, unevenly distributed, occasionally in small clusters; vascular hyphae not observed. LAMELLA EDGE TISSUE: sterile.

BASIDIOSPORES: [558/27/16] (7.5–) 8.0–11.0 (–13.6) × (5.7–) 6.5–8.4 (–9.9) µm, (**L** = (8.3–) 8.7–10.0 (–10.3) µm; **L'** = 9.4 µm; **W** = (6.6–) 6.9–7.7 (–7.8) µm; **W'** = 7.3 µm; Q = (1.06–) 1.15–1.43 (–1.73); **Q** = (1.22–) 1.24–1.37 (–1.40); **Q'** = 1.29), hyaline, colorless, thin-walled, smooth, inamyloid, broadly ellipsoid to ellipsoid, rarely elongate, usually at least somewhat adaxially flattened, wand-like or very narrowly clavate in early development;

apiculus sublateral (or rarely lateral in immature material), cylindric; contents monoguttulate to multiguttulate, with or without small additional granules; white in deposit.

ECOLOGY: Costa Rica: Solitary to subgregarious, at 1000–2500 m elev. In mixed forest with, and sometimes dominated by, *Quercus* (including *Q. brenesii*, *Q. copeyensis*, *Q. oocarpa*, and *Q. seemannii*), with or without substantial understory. Honduras: In undisturbed *Quercus* forest.

MATERIAL EXAMINED: **COSTA RICA**: ALAJUELA—Bosque del Niño, 31.v.1996 R.E. Halling & J.L. Mata [Halling 7591] (NY; USJ). CARTAGO—Estrella, 28.vii.1992 B.A. Strack & G.M. Mueller [Mueller 4433] (F 1102486), 4.vi.1996 R.E. Halling & J. Ammirati [Halling 7603] (NY; USJ); Tapanti, 20.vi.2001 R.E. Halling & J. Carranza [Halling 8198] (NY; USJ). GUANACASTE—Cacao no. 2, 4.vi.1994 J.P. Schmit 475 (F; USJ). SAN JOSÉ—La Chonta, 11.vii.1982 L.D. Gómez 18172 (F 1100529), 7.vi.1994 R.E. Halling & T.J. Baroni [Halling 7237] (NY; USJ), 16.vi.1995 K. Shanks, R.E. Halling, R.E. Tulloss & R.H. Petersen [Tulloss 6-16-95-C] (RET 333-6; USJ), R.E. Tulloss 6-16-95-D (RET 332-7; USJ), R.E. Halling s.n. [Tulloss 6-16-95-K] (RET 337-1; USJ), 11.vi.2001 R.E. Halling, B. Buyck, R. Aldana-Gómez [Halling 8178] (NY;USJ); El Empalme, 15.vi.1995 R.E. Halling, R.H. Petersen, K. Shanks, R.E. Tulloss [Tulloss 6-15-95-H] (RET 333-2; USJ); Jardín de Dota, 13.vii.1993 G.M. Mueller 4501 (F 1110801); San Gerardo de Dota no. 1, 8.vi.1994 R.E. Halling s.n. [G.M. Mueller 4659] (F 1112080; USJ), 21.vi.1995 R.E. Tulloss 6-21-95-G (holotype, USJ; isotype, NY), 24.vi.1997 R.E. Halling, G.M. Mueller, S. Huhndorf & D. Quist [Halling 7737] (NY; USJ). **HONDURAS**: FRANCISCO MORAZAN—Tegucigalpa – P. N. La Tigre, Sendero Bosque Nublado, 4.vii.1991 G.M. Mueller, B.A. Strack, R. & M. Singer & R. Andino [Mueller 4119] (F 1098650).

COMMENTS — The (a) relatively shallow subhymenium; (b) subhymenial base dominated by elongate, curved, intercalary cells; (c) plentiful clamps at bases of basidia; (d) habit; (e) proportionately long marginal striations; (f) strongly pigmented pileus and stipe decoration; and (g) broadly ellipsoid to ellipsoid spores indicate that this entity has strong affinity to the group of taxa phenetically similar to *A. hemibapha* (Berk. & Broome) Sacc. (Saccardo 1887), *A. jacksonii* Pomerl. (Pomerleau 1984), *A. arkansana* H.R. Rosen (Rosen 1926), *A. hayalyuy* Arora & Shepard (Shepard et al. 2008), etc.—taxa of *Amanita* stirps *Hemibapha* (Tulloss 1998a, 2009a).

The species of the above cited group that is most similar to *A. garabitoana* in habit and in size and shape of spores is *A. arkansana* the known range of which lies within the southeastern United States (Arkansas to the Gulf Coast states—from Texas to Florida). The present taxon differs from *A. arkansana* in at least the following:

- Pigmentation: The North American species lacks olivaceous tints in the pileus at all stages of development and is not as deeply pigmented as is

the red-brown disc of *A. garabitoana*; moreover, *A. arkansana* retains its yellow-orange coloration as dried rather than becoming dark brown to sordid or olivaceous tan. In addition, the patches on the stipe of *A. arkansana* are concolorous with the stipe (yellow) at first and become deeper yellow or more orange from handling; they are never olivaceous or sordid yellow.

- Fragile outer part of pileus: The basidiome of *A. arkansana* is notoriously fragile and difficult to collect without breaking, which is not the case for *A. garabitoana*. The portion of pileus context with greater thickness than a membrane extends half or less of the radius toward the margin in examined material of *A. arkansana*, but 75% to 85% of the radius in *A. garabitoana*.
- Fragility of the stipe: The ratio of the diameter of the central cylinder to the diameter of the stipe (both measured at midstipe) in specimens of the two taxa are 0.5–0.56 for *A. arkansana* and 0.21–0.42 (–0.52) for *A. garabitoana*. Hence, the context forms a supporting wall with thickness of 22%–25% of the stipe diameter in the northern species and with (24%–) 29%–40% of the stipe diameter in the mesoamerican species.
- Tyrosinase spot test: In contrast to the strong positive reaction for tyrosinase in *A. garabitoana*, that reaction is very limited in most of the context of *A. arkansana*. In immature material of *A. arkansana*, using paracresol, a positive reaction for tyrosinase was only seen in the limb of the volva and in five, small and widely scattered spots on the stipe and pileus context after 18 min. Another test with more mature material, using L-tyrosine (only on pileipellis, stipe surface, and stipe context), produced very faint positive reaction on the stipe surface and pileipellis after 19 min. Voucher specimens: Tulloss 10-26-85-A and 7-16-87-C.
- Spore size: Spores of *A. arkansana* are somewhat smaller than those of *A. garabitoana*: [290/13/7] (7.0–) 7.7–10.5 (–15.0) × (5.6–) 6.0–8.0 (–10.2) µm, (**L** = (8.0–) 8.3–9.9 µm; **L'** = 9.0 µm; **W** = (6.3–) 6.5–7.3 µm; **W'** = 6.9 µm; Q = (1.10–) 1.19–1.43 (–1.70); **Q** = (1.22–) 1.24–1.38; **Q'** = 1.30).

MATERIAL EXAMINED (*Amanita arkansana*): **U. S. A.**: ARKANSAS—Pulaski Co. – Little Rock, Arkansas Dept. of Pollution Control & Ecology, 14.vi.1994 J. Justice s.n. (RET 136-6). Washington[?] Co. – E of Fayetteville, E of Mt. Sequoia, 13.x.1925 [packet marked "13.x.1926" (sic)] H.R. Rosen s.n. (holotype[1], BPI; isotypes TENN 21294 & 21299). FLORIDA—Alachua Co. – Gainesville, across from Florida St. Mus., 11.viii.1985 A. Norarevian s.n. [Tulloss 8-11-85-AN1] (RET 137-5). MISSISSIPPI—Jackson Co. – Pascagoula R. Wildlife Mgt. Area, 16.viii.1987 D.C. & R.E. Tulloss 7-16-87-C (in herb. David T. Jenkins, Univ. Ala., Birmingham; RET 149-5). MISSOURI—Stoddard Co. – Mingo Nat. Wildlife

Refuge, 19.ix.2008 J. Justice s.n. (RET 450-8). TEXAS—Hardin or Tyler Co. – Big Thicket Nat. Preserve, Turkey Crk. Unit, 26.x.1985 A. Norarevian & J. Justice s.n. [Tulloss 10-26-85-A] (RET 139-10); Tyler Co. – 8 km E of Spurger, Forest Lake Exp. For., off rd. FM1013, ca. plots 39 & 41, 26.vi.1994 D.P. Lewis 5302 (RET 283-5).

So far as is known (C. Bas pers. corresp.; Tulloss unpub. data), it is not unusual to find vascular hyphae especially plentiful in the pileus context above the stipe in *Amanita*. This is an item worthy of further study. Bas (1969 and pers. corresp.) has noted that vascular hyphae are sometimes concentrated in damaged areas in *Amanita*. Since they arise from filamentous undifferentiated hyphae (Tulloss unpub. data), it may be the case that they are produced in response to damage or in areas of mechanical stress (such as the stipe-pileus convergence region). One hypothesis might be that the (partially?) insoluble material often seen when a vascular hypha is cut or broken during sectioning may include an antibiotic or some other aid to maintaining the integrity of the basidiome and, hence, reproduction of the species.

We also wish to make it clear that we make no claim for novelty with regard to the arrangement of acrophysalides in the center of the pileus. However, it does seem of interest to examine (in the future) the mechanical structure(s) by which the joining of the stipe and cap takes place in *Amanita*.

Mueller 4501 was immature when dried; in measuring spores from a single mount, three 5-sterigmate basidia were noted with the sterigmata bearing content-less, wand-like or very narrowly clavate spores. The single basidiome of this collection is considered by us to be bearing abnormal spores.

Mueller 4119 was immature when dried.

2. Section *Lepidella*

2.1. Key to section *Lepidella* in Costa Rica and Honduras — This key treats only those subsections of section *Lepidella* represented in the region of study. Species marked by an asterisk (*) are not treated at length in this paper.

1. Pileus pinkish buff; universal veil on pileus in tightly appressed patches, white to concolorous, comprising outer layer of interwoven filamentous undifferentiated hyphae and interior dominated by chains of narrow, relatively large, inflated cells with periclinal orientation, pileipellis poorly developed; spores (7.8–) 8.2–10.0

1. While there are three collections representing parts of the original collection, none is marked as the holotype. The place of deposit of the holotype was not provided in the protolog. BPI was indicated as the location of the "type" by Gilbert (1940-41: 98, caption for Tab. IX, fig. 2). This is fortunate because the portion of the type in BPI has the most thorough and abundant representation of all parts of the included basidiomes of any of the three duplicates of the original collection.

(–10.5) × (5.5–) 6.0–7.0 (–8.0) µm, with **Q** = 1.34–1.39 (*Amanita* subsect. *Vittadiniae* Bas) .....

A. species HON1*.

1. Universal veil as warts or irregular patches or as pyramidal warts; elements comprising these warts disordered or having more or less anticlinal orientation, never as chains of relatively large, narrow, inflated cells with strong periclinal orientation.

2. Outer layer of volva consisting mainly of hyphae; consequently, often with small, incomplete volval limb on stipe's basal bulb; lamellae ochraceous to yellow to pale orange. (*Amanita* subsect. *Gymnopodae* Bas).....

3. Spores (7.8–) 8.1–10.0 (–11.5) × (5.3–) 6.0–7.5 (–8.7) µm, with **Q** = 1.33–1.41 (–1.44); found at 1600–1720 m elev. in Prov. Cartago with *Quercus oocarpa* and *Q. seemannii* .....

A. conara.

3. Spores (6.1–) 7.0–9.5 (–11.6) × (5.1–) 6.9–7.9 (–9.5) µm, with **Q** = (1.11–) 1.15–1.23; found at 1350–1500 m elev. in Prov. Puntarenas with *Q. corrugata* and *Q. seemannii* .....

A. species CR18*.

2. Volva never submembranous, never forming limb at base of stipe; lamellae never ochraceous (*Amanita* subsect. *Solitariae* Bas).

4. Pileus rat gray to brown, micaceous gray to pallid in age or after rains wash away pigment, with brownish gray pulverulent universal veil remains in pyramidal warts or irregular patches, often darker than pileipellis; bulb of stipe napiform to radicating, often markedly radicating; spores (7.5–) 8.5–13.2 (–16.0) × (5.1–) 6.0–8.5 (–12.6) µm, with **Q** = 1.35–1.68 (–1.70) .....

A. costaricensis.

4. Pileus and universal veil white, at least at first.

5. Much of basidiome staining ochraceous buff to rust colored to chestnut brown or yellow to cinnamon; universal veil on pileus as flattened to slightly erumpent large warts or small patches; all parts of basidiome liable to staining pale yellowish and, eventually, cinnamon; bulb of stipe napiform, never abrupt or subabrupt, decorated with narrow rings of volval tissue beginning on lower stipe above bulb and continuing to approximately broadest part of bulb; spores (8.5–) 9.0–12.5 (–14.0) × (6.5–) 7.0–9.5 (–10.2) µm, with **Q** = 1.29–1.32 .....

*A. advena**.

5. Pileus not staining, often covered rather densely by minute pyramidal warts; bulb slenderly subnapiform to napiform, subradicating, with either rings of flocculence or minute pyramidal warts around top of bulb; spores (7.0–) 9.1–13.0 (–17.5) × (5.2–) 5.9–7.8 (–9.5) µm, with **Q** = (1.35–) 1.50–1.81 .....

*A. polypyramis**.

2.2. Descriptions of taxa —

Amanita conara Tulloss & Halling **sp. nov.** FIGS. 5–8

MYCOBANK MB 518296

Pileus 60–150 *mm latus, subochraceobubalinus vel subbubalinus vel aurantiocremeus vel griseoaurantius, labibus subaurantiis vel subbrunneis, margine longistriato, nonappendiculato, reliquiis veli universalis verrucas vel assumenta formanti. Lamellae liberae, subconfertae vel confertae, subcremeae vel subaurantio-flavobubalinae; lamellulae subattenuatae vel rotundato-truncatae vel truncatae. Stipes* 71–115^{+} × 11–30$^{\pm}$ *mm, albus vel subroseus vel subbrunneoroseus, annulatus, bulbo robusto* 42–60 × 37–60 mm, *interdum velo universali membranaceo pertenui super bulbum. Basidiosporae* (7.8–) 8.1–10.0 (–11.5) × (5.3–) 6.0–7.5 (–8.7) µm, (**L** = (8.6–) 8.8–9.4 µm; **L'** = 9.1 µm; **W** = 6.5–6.8 (–7.0) µm; **W'** = 6.6 µm; **Q** = (1.17–) 1.23–1.51 (–1.70); **Q** = 1.33–1.41 (–1.44); **Q'** = 1.37), *amyloideae, late ellipsoideae vel ellipsoideae. Basidiae* 30–58 × 9.1–13 µm. *Fibulae relative communis. In quercetis tropicalibus montanis costaricensibus habitat. Species Amanitae subsectionis Gymnopodae simulanissima. Species nova ob distributionem geographicam, magnitudinem et formam sporarum, consortionem Querco, colorem et formam et anatomiam microscopicam veli universalis et limbi interni, et absentiam rhizoidei bulbo stipitis.*

HOLOTYPE — Costa Rica, Prov. Cartago, Estrella, 14.vi.1996 R.E. Halling & J. Ammirati [Halling 7686] (USJ).

ETYMOLOGY — κοναρος (Grk.), fat or well-fed, because of the large basal bulb of the present species; proposed English name, "Fat Barefoot Lepidella."

PILEUS: 60–150 mm wide, light ochraceous buff (ca. 5A2) with light orange stains (ca. 6A4) or pale buff to faintly tannish or orangish cream (paler and more tan than 7.5YR 8/4, paler than 10YR 8/4) or grayish orange (6B4), occasionally stained faintly brown here or there, convex at first, planoconvex and somewhat undulate at maturity, not viscid when wet, dull at first, later shiny when dry; *context* white to cream to slightly brownish cream, context unchanging or slowly staining brown to reddish brown when cut or bruised, 7–15 mm thick at stipe, thinning evenly and slowly to margin, rather thick even near margin; *margin* nonstriate, decurved, appendiculate (at first with appendiculate material floccose and pale pinkish, at maturity only with scattered crumb-like bits); *universal veil* as crumb-like warts or pyramidal warts over disc, sometimes as eccentric patches, at first pale orangish white to pinkish (5A2, paler toward pileus margin) becoming sordid or brown or taking on brownish tint, smooth surfaced, often easily taking fingerprint, pulverulent to subfelted, detersile, with patches having membranous upper layer.

LAMELLAE: free, with decurrent lines on stipe apex connecting to striations on upper surface of partial veil, close to subcrowded to crowded, pale cream (immature) to slightly orangish yellowish tan in mass, more vividly colored in

Fig. 5. *Amanita conara*. Habit (Halling 7272) (×0.45). Photo by R E. Halling.

side view [light cream in immature material, then more yellow than 2.5Y 7/6 or more brown than 2.5Y 8/6 or more orange than 4B6 or more brown than 4A6 or pale yellow (4A4) or pale orange (5A3)], unchanging when cut or bruised, 6.5–12 mm broad, occasionally forked, with edge minutely pulverulent and white to approximately concolorous with partial veil; *lamellulae* subattenuate to rounded attenuate to truncate, unevenly distributed, of diverse lengths, plentiful.

STIPE: 71–115^{+} × 11–30$^{\pm}$ mm, white, sometimes with ochraceous to light orange stains, sometimes faintly brick colored or pale pink after handling or in wounds, narrowing upward, flaring at apex, pulverulent above partial veil, sometimes satiny below partial veil, longitudinally striatulate, with upward pointing fibrillose scales or squamules [concolorous to light orange (ca. 6A4), with tips sometimes faintly brick colored or concolorous with partial veil and limbus internus of universal veil], with shaggy region beginning below annulus and ending roughly 10 mm above bulb; *bulb* 42–60 × 37–60 mm, robustly clavate-subnapiform to robustly napiform, with white mycelial threads prominent at very base; *context* solid or stuffed? (possibly appearing so due to larval damage), concolorous with pileus context, with bruising/staining reaction as in pileus context, with larval tunnels yellowish in center of stipe and reddish brown elsewhere; *partial veil* apical, submembranous, shredding, sometimes deciduous at least in part, pale orangish white (5A2), with underside bearing

Fig. 6. *Amanita conara*. Habit (Tulloss 6-13-95-C) (×0.74). Photo by R.E. Tulloss.

warts and flocculence, with upper side striate, sometimes thickened at edge; *universal veil* often present as short thin limb and/or in incomplete and sometimes slightly overlapping rows of triangular flaps (minute, weakly structured, upward-pointing) 0.5± mm high on (or appressed to) upper part of bulb, with edges of such structures sometimes appearing frayed and darkening with age (viewed at 6–12×), often with nearly complete limbus internus partially encircling (about half-circumference) base of stipe and in other small fragments on lower stipe above bulb, 1–2 mm thick, nearly white to pale orangish white (5A2).

Odor "earthy" in immature specimen; rich, strong, like "old ham" (but sometimes not unpleasantly) or like "unwashed athletic socks" in mature material. *Taste* not recorded.

MACROCHEMICAL TESTS: Spot test for laccase (syringaldazine): negative throughout basidiome. Spot test for tyrosinase (paracresol): positive in stipe (excluding bulb) and pileipellis and often in large areas of lamellae and pileus context (outside of disc) or strongly positive throughout basidiome except for parts of lamellae. Test vouchers: Halling 7272, Mueller 4643, Tulloss 6-13-95-C.

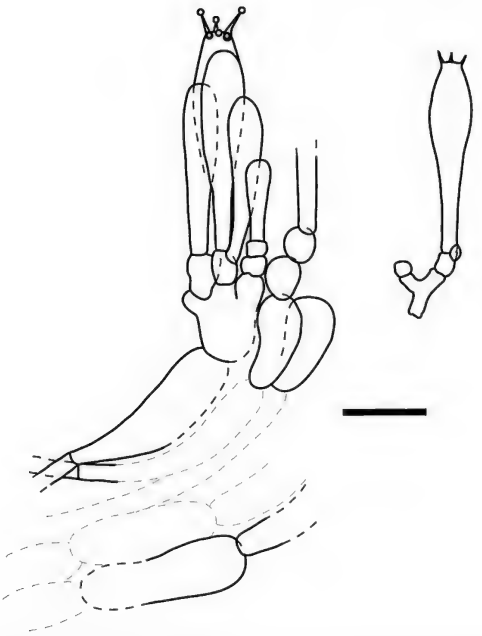

Fig. 7. *Amanita conara* (holotype). Elements of hymenium and subhymenial tree (gray lines of subhymenial base are semischematic). Scale bars = 20 µm.

PILEIPELLIS: narrow and with pronounced boundaries, bounded above by densely placed anticlinally oriented hyphae binding upper surface to remains of universal veil, bounded below by loosely interwoven and disordered tissue of pileus context, with separation from universal veil at first via mechanical rupture of hyphae; 30–55 µm thick at approximately midradius in mature or near mature material, lacking substantial suprapellis, with gelatinization absent even at point of breaking of partial veil and in mature material limited to surface, with elements densely packed vertically, yellow-orange; filamentous undifferentiated hyphae 2.5–7.5 µm wide, branching, disordered and interwoven over disc, dominantly subradially arranged with some criss-crossing hyphae away from disc, often yellow or yellowish, hyphal tips of broader hyphae common; vascular hyphae 3.4–11.4 µm wide, sinuous, sometimes in tangles, yellow, scattered. PILEUS CONTEXT: filamentous undifferentiated hyphae 2.5–8.9 µm wide, occasionally branching, sometimes fasciculate, common in disc, plentiful at mid-radius; acrophysalides broadly ellipsoid to ellipsoid (up to 80 × 60 µm in disc) or clavate (up to 131 × 48 µm), similarly shaped and smaller at mid-radius, commonly with inflated subterminal cell, in disc dominating and disordered, at mid-radius plentiful and with tendency to

subradial orientation; vascular hyphae 2.8–17.8 µm wide, sinuate to hypersinuate, rarely branching, common in region immediately below pileipellis, rather common elsewhere, scattered in all mounts. LAMELLA TRAMA: bilateral, divergent; w_{cs} = 50–65 µm (good rehydration); central stratum comprising interwoven filamentous undifferentiated hyphae 2.5–11.4 µm wide, lacking inflated intercalary segments; subhymenial base dominated by densely packed divergent filamentous undifferentiated hyphae 3.8–11.4 µm wide, with angle of divergence shallow; terminal, inflated cells not observed; vascular hyphae 2.3–8.5 µm, infrequently branching, sinuous, uncommon. SUBHYMENIUM: w_{st}-near = 70–85 µm (moderate to good rehydration); w_{st}-far = (80-) 90–105 µm (moderate to good rehydration); scant, often with only one (or no) cell(s) between bases of longest basidia/-oles and subhymenial base, with basidia arising from elongate cells of subhymenial base and subglobose to branched to ellipsoid to clavate cells and uninflated hyphal segments, with such elements arranged approximately perpendicular to central stratum. BASIDIA: (30–) 34–59 (–60) × 9.1–13.0 (–14.4) µm, 4-sterigmate, with sterigmata up to 7.5 × 2.0 µm and sometimes subcylindric; clamps and proliferated clamps relatively common. UNIVERSAL VEIL: *On pileus, exterior surface*: particularly well-formed and in place on surface of patches, very thin, comprising lattice of irregularly ordered fascicles of partially gelatinized orange-brown hyphae, with fascicles up to 18 (or more) hyphal diameters wide; filamentous undifferentiated hyphae 1.8–3.9 µm wide, branching; with immediately underlying thin membranous layer, comprising loosely interwoven filamentous undifferentiated hyphae 2.2–8.9 µm wide, disordered, commonly branching, anastomosing, plentiful to dominating, sometimes in narrow fascicles, thin-walled, colorless and hyaline to (infrequently) yellowish and subrefractive; inflated cells uncommon, terminal, singly or in pairs, broadly clavate to clavate to cylindric, thin-walled, hyaline, up to 57 × 13.0 µm; vascular hyphae not observed. *On pileus, interior*: all elements strongly vertically aligned, orangish brown on wart surface; filamentous undifferentiated hyphae 1.0–12.1 µm wide, frequently branching, occasionally anastomosing, inside wart colorless and hyaline or (occasionally) yellowish and with subrefractive walls; inflated cells in terminal chains of up to at least five, thin-walled, plentiful, colorless and hyaline inside wart, barrel-shaped to ovoid to broadly clavate to clavate to narrowly fusiform and up to 48 × 25 µm in lower third of wart above basal part and subglobose to ellipsoid to clavate (up to at least 78 × 38 µm) to narrowly clavate or narrowly fusiform (up to 91 × 19.0 µm) to irregularly shaped and sometimees connected with three or more hyphae via separate septa in upper third of wart; vascular hyphae not observed except in upper part of wart and there very infrequent and (e.g.) 2.2–4.9 µm wide. *On pileus, basal part*:

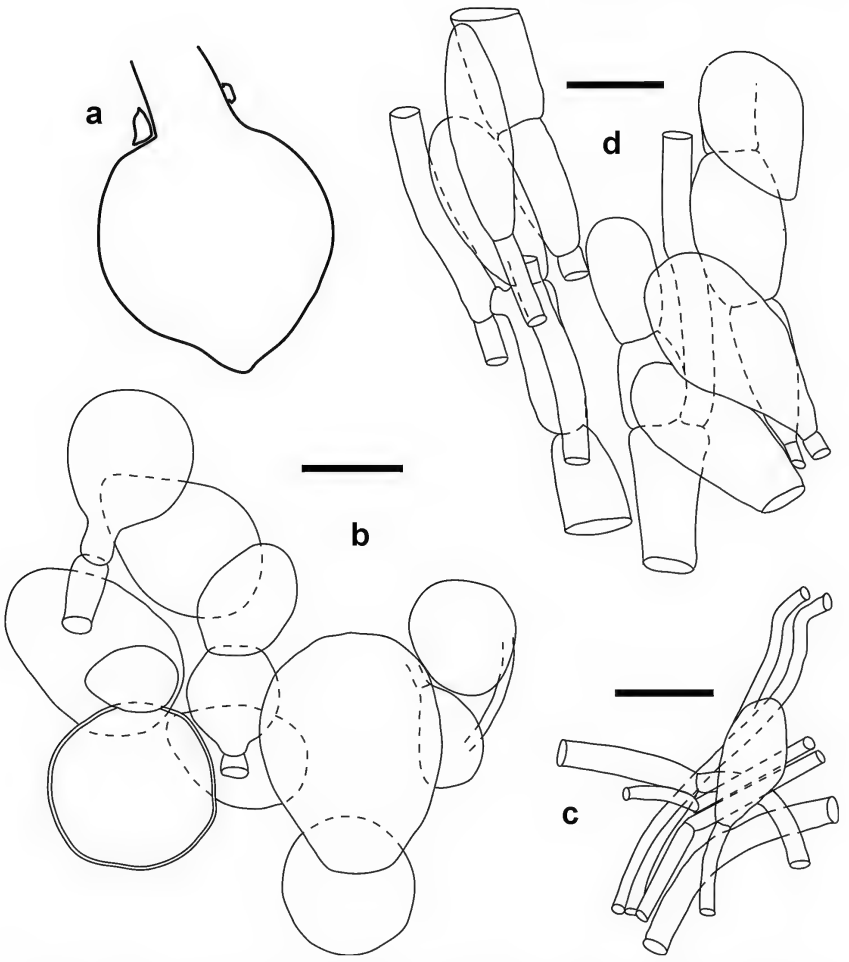

Fig. 8. *Amanita conara.* a. Cross-section of fragments of limbus internus on bulb and lower stipe (Tulloss 6-13-95-C) (×0.82). b. Elements of universal veil (limbus internus) viewed through—from above—remains of partial veil (not shown) (holotype). c. Elements of universal veil, just below gelatinized hyphal layer on top of pileal wart (Halling 7272). d. Elements of universal veil near base of pileal wart (Halling 7272). Scale bars = 20 μm.

densely packed, anticlinally aligned hyphae connecting intimately with pileipellis. *On underside of partial veil* (i.e., limbus internus): filamentous undifferentiated hyphae 2.2–10.8 μm wide, occasionally branching, common; inflated cells dominating, terminal singly or as concatenated pairs, globose to subglobose to pyriform to broadly ellipsoid to ellipsoid to elongate to broadly

clavate to clavate to narrowly clavate (up to 60 × 33 µm), dominantly thin-walled, occasionally with wall up to 0.8 µm thick; vascular hyphae not observed. *On stipe base*: with structure very like that of surface layers of universal veil on pileus; filamentous undifferentiated hyphae dominating, thin-walled, slightly gelatinized at surface, hyaline and colorless or (occasionally) with walls yellowish and subrefractive, 2.5–10.0 µm wide, commonly branching, fasciculate, densely packed, with predominantly vertical orientation (i.e., not disordered as on pileus); inflated cells rather infrequent, scattered, terminal, narrowly clavate to clavate (e.g., 30^+ × 12.5 µm); vascular hyphae not observed; clamps not observed. STIPE CONTEXT: longitudinally acrophysalidic; filamentous undifferentiated hyphae 3.2–17.8 µm wide, branching occasionally, plentiful in interior, dominant near surface, sometimes constricted at septa; acrophysalides up to at least 395 × 32 µm, plentiful in interior, common near surface; vascular hyphae 7.6–11.4 µm wide, sinuous, infrequent, occasionally clustered; clamps not observed. PARTIAL VEIL: very thin and sparse layer of criss-crossing, hyphae, in mounts dominated by inflated cells of limbus internus attached to underside of hyphal framework; filamentous undifferentiated hyphae 1.5–3.8 µm wide, occasionally branching, in narrow fascicles, rapidly gelatinizing prior to maturity of basidiome; inflated cells not observed; vascular hyphae not observed. LAMELLA EDGE TISSUE: sterile.

BASIDIOSPORES: [181/8/4] (7.8–) 8.1–10.0 (–11.5) × (5.3–) 6.0–7.5 (–8.7) µm, (**L** = (8.6–) 8.8–9.4 µm; **L'** = 9.1 µm; **W** = 6.5–6.8 (–7.0) µm; **W'** = 6.6 µm; Q = (1.17–) 1.23–1.51 (–1.70); **Q** = 1.33–1.41 (–1.44); **Q'** = 1.37), hyaline, colorless, thin-walled, smooth, amyloid, broadly ellipsoid to ellipsoid, sometimes swollen at one end, often at least somewhat adaxially flattened; apiculus sublateral, cylindric; contents multi- to monoguttulate; color in deposit unknown.

ECOLOGY: Subgregarious to scattered, at 1600–1720 m elev. In thick, dark, wet loam under shallow layer of leaf litter of mixed broadleaf forest including *Quercus oocarpa* or in such a habitat with that tree and *Q. seemannii*.

> MATERIAL EXAMINED: **COSTA RICA**: CARTAGO—Estrella, 14.vi.1996 R.E. Halling & J. Ammirati [Halling 7686] (holotype, USJ; isotype, NY); off Interamerican Hwy., 6 km S of Cartago at rd. to Palo Verde, 28.vii.1992 B.A. Strack & G.M. Mueller [Mueller 4434] (F 1102487); Palo Verde, 31.v.1994 G.M. Mueller 4643 (F 1112041; USJ), 11.vi.1994 R.E. Halling & T.J. Baroni [Halling 7272] (NY; USJ), 13.vi.1995 R.E. Tulloss 6-13-95-C (RET 153-4; USJ).

COMMENTS — Four taxa have been assigned to *Amanita* subsect. *Gymnopodae* world wide, but all can be easily distinguished from the present species. In addition to their general habit, three of these species have in common with *A. conara* a number of distinctive characters among which are rubescent or dark-

ening context, a distinctive odor, yellow or orange tints on the lamellae, and a submembranous surface layer of the universal veil.

- *Amanita gymnopus* Corner & Bas (Corner & Bas 1962), described from Malaysian "jungle" and reported from southern China (Chen et al. 2001, Yang 2005) and Japan (Hongo 1982), has small (5–7 × 5–6.5 µm per Corner and Bas), globose to subglobose spores; a universal veil that forms submembranous patches and not warts on the pileus; and marked rhizoids attached to the stipe base (Bas 1969: fig. 378). Chen et al. reported spores of 6.0–7.5 (–8.0) × (5.0–) 5.5–6.5 µm from a single specimen. Yang reported spore data as follows in specimens from at least three localities: 6.0–8.5 × (5.0–) 5.5–7.5 µm, (Q = (1.03–) 1.07–1.24 (–1.42); **Q** = 1.14 ± 0.08).[1]
- *Amanita ochraceobulbosa* A. E. Wood (Wood 1997) has subglobose to broadly ellipsoid spores 9.3–11.7 × (7.3–) 8.1–9.6 (–10.2) µm with est. **Q** = 1.2, a cap that is cream to cream-buff to yellow-orange-cream with universal veil remains as (often large) pyramidal warts, gills that are white to cream, flesh that apparently is nonstaining when cut or bruised, and pyramidal warts sometimes present on the stipe's basal bulb. Absence of a submembranous outer volval layer, presence of warts on the bulb, and no mention of staining flesh or distinctive odor suggest that this taxon is not appropriately placed in subsect. *Gymnopodae*.
- *Amanita ochrophylla* (Cooke & Massee) Cleland (Cleland 1924), described from edges of open forest in eastern and southeastern Australia (Bas 1969: 556), has larger, proportionately narrower spores (9–11 × 5.5–7 µm) with Q = 1.3–2.0 and **Q** = 1.5–1.8; a limbus internus that is apparently more coherent than that of *A. conara* and is frequently left as a "second annulus" on the stipe just below the partial veil (Bas 1969: fig. 374); flesh that becomes pink on exposure; etc. Wood (1997: 803) gives spore measurements as follows: 9.3–10.8 (–12.6) × 5.4–7.4 (–8.4) µm, with **Q** = 1.43–1.72 (–1.88). We estimate **Q'** for Wood's material as 1.55–1.6.
- *Amanita ochrophylloides* D. A. Reid (Reid 1978, 1980), described from "under *Eucalyptus* sp. (of peppermint group)" in the state of Victoria, Australia, has predominantly subglobose to broadly ellipsoid to ellipsoid spores (7.0–10.0 × 5.0–8.0 µm, with est. **Q** = 1.3); a pale brown pileus with dark, "strobiliform" warts; a tendency to darken from handling; pale golden yellow lamellae; a slight volval limb on the

1. Yang's Q corresponds to the Q of our notation; and his **Q**, to our **Q'**.

Fig. 9. *Amanita costaricensis*. a. Habit (holotype) with weathered cap on right (×0.55). b. Habit (Tulloss 6-23-95-M) (×0.37). Photos by R. E. Tulloss.

stipe's basal bulb; an odor that is faint and mealy; abundant, highly refractive hyphae in the universal veil on the pileus; etc.

Guzmán (1975) reported *A. ochrophylla* from *Quercus* forest in Guerrero. His macroscopic description fits *A. conara* rather well; and his measurements for spores are 9–11 × (5.5–) 6.0–7.5 µm. This is a relatively good match for our spore data from *A. conara*. We estimate a **Q'** of approximately 1.5 for spores of Guzmán's material. We have not yet reviewed the material Guzmán cited, but it appears to us that *A. conara* may occur in Mexico.

Mueller 4643 consists of a single, rather large, immature specimen of *A. conara*. It comes from the same habit as the other material cited and is easily recognizable because of excellent photographs and notes on fresh material that accompany the exsiccatum; moreover, the thin membranous outer layer of the universal veil can be seen on scalp sections of the universal veil remnants on the pileus.

We have three collections of what appears to be a similar, but possibly distinguishable, taxon from Ctn. Coto Brus, Prov. Puntarenas (La Amistad sites nos. 1 and 2) in association with *Q. corrugata*, *Q. seemannii,* and other oaks and belonging in subsection *Gymnopodae* ("species CR18" in the above key).

These collections differ from *A. conara* in colors of the basidiome (not all collections are fully annotated) and consistently have smaller subglobose to broadly ellipsoid spores: [160/8/3] (6.1–) 7.0–9.5 (–11.6) × (5.1–) 5.9–7.9 (–9.5) µm, (**L** = 7.6–8.2 (–8.3) µm; **L'** = 7.9 µm; **W** = (6.3–) 6.7–7.0 µm; **W'** = 6.7 µm; Q = (1.04–) 1.08–1.29 (–1.41); **Q** = (1.11–) 1.15–1.23; **Q'** = 1.17). All three collections are deposited in NY (Halling 7803, 8148. 8360).

The present species was previously called "*A.* species CR10" in drafts and keys circulated by Tulloss.

Amanita costaricensis Tulloss, Halling, G.M. Muell. & Singer **sp. nov.** FIGS. 9–11
MYCOBANK MB 518297

Pileus (43–) 50–120 (–145) *mm latus, brunneus vel alutaceus vel griseus, aliquando subroseotinctus, disco fuscior, hemisphaericus vel convexus demum applanatus disco subumbonato; contexto albo, aliquando subochraceo ubi laeso; margine appendiculato aliquando brevistriato; velo universali griseo vel brunneo vel atrobrunneo verrucato. Lamellae liberae vel subadnatae, confertae, saepe subventricosae,* 3–7.5 *mm latae, lamellulis truncatis vel attenuatis. Stipes* 52–124 × 9–18 (–22) *mm, subalbus vel griseus, annulatus, pulverulentus vel flocculosus sub annulo; bulbo* (30–) 57–112+ × 17–40 *mm, napiformi vel dauciformi, radicanti; velo universali pulverulento vel verrucato, fuligineogriseo vel concoloro. Sporae amyloideae,* (7.5–) 8.5–13.2 (–16.0) × (5.1–) 6.0–8.5 (–12.6) *µm,* (**L** = (9.1–) 9.4–12.3 *µm;* **L'** = 10.8 *µm;* **W** = 6.4–7.7 (–8.3) *µm;* **W'** = 7.2 *µm;* Q = (1.10–) 1.27–1.83 (–2.15); **Q** = 1.35–1.68 (–1.70); **Q'** = 1.51), *late ellipsoideae vel ellipsoideae vel elongatae. Fibulae vulgaris. In quercetis tropicalibus montanis costaricensibus et hondurensibus habitat. Amanita onusta et A. atkinsoniana Amanitae stirpis Microlepis simulanissimae. Species nova ob magnitudinem sporarum, magnitudinem basidiomae, lamellarum non flavescentem et non celeriter putrescentem, et ubi probata paracresolo non reagens.*

HOLOTYPE — Costa Rica, Prov. San José, Ctn. Dota, 21.vi.1995 R.E. Halling s.n. [Tulloss 6-21-95-M] (USJ).

ETYMOLOGY — In honor of the Republic of Costa Rica.

PILEUS: (43–) 50–120 mm wide [up to 145 mm in Mueller 4368], brown (6C4-5) to light brown (5-6D4-5) to light brown with slight pinkish cast (ca. 7D5) to pale sordid tan to yellowish cream (then with sordid tan disc) to light brownish gray (7.0YR 5.4/1.2) to brownish gray to mouse (or rat) gray, micaceous gray in old material (Gómez & Alfaro 20641) or dull dark brown over disc [Mueller 4368], with pigment at least sometimes washed out by rain, hemispheric to convex at first, becoming applanate around subumbonate disc, tacky when moist, sometimes viscid over disc, dull to satiny to subshiny, shiny in age, sometimes becoming areolate and then with areolae centered on warts; *context* white, usually with pale brownish gray to gray to brown region under pileipellis, sometimes pale ochraceous when exposed, 3–10.5 mm thick over stipe, thinning evenly to margin; *margin* nonstriate, sometimes short sul-

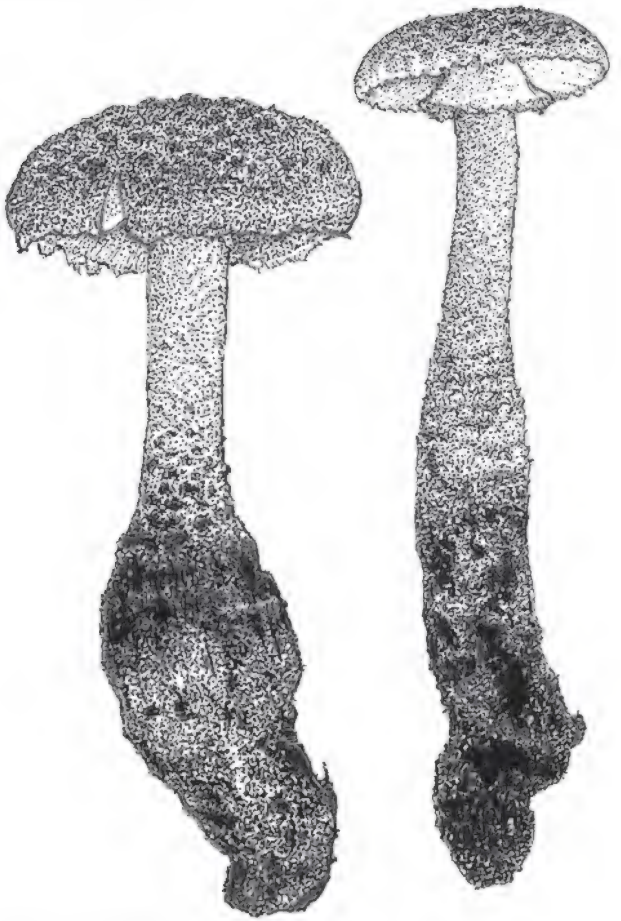

Fig. 10. *Amanita costaricensis*. Habit (Tulloss 6-23-95-M) (×0.63).

cate (0.05R) in age, incurved at first, then decurved, occasionally uplifted in age, appendiculate with white and or gray floccose-felted shreds of partial veil; *universal veil* as patches or flakes or warts (some rather broad and irregular, some small and pyramidal), often squamulose near margin, gray to brownish gray to brown or dark grayish brown and darker than pileus (6D-E4-6 to 7E6 to 7F7 to 5.6YR 3.4/0.9), occasionally as flat pinkish brown (7B4) patches, pulverulent, darkening with age, verruculose (10× lens), detersile.

LAMELLAE: free to narrowly adnate, close to subcrowded to crowded, lacking decurrent lines on stipe apex, white to pale cream in mass, off-white to slightly sordid white to very pale yellowish white in side view, reddish brown where damaged, relatively thick, 3–7.5 mm broad, frequently subventricose, entire, with thin gray edge; *lamellulae* truncate to rounded truncate to subattenuate to attenuate to attenuate in steps, plentiful, unevenly distributed, of diverse lengths or all very short [only reported for Mueller 4368].

STIPE: 52–124 × 9–18 (–22) mm [broadest in luxuriant mature specimen of Mueller 4368], ground color white to off-white to pale grayish, pallid to gray matte to whitish-gray and smooth to subglabrous above partial veil, below partial veil (and extending downward to first row of warts of universal veil) decorated with densely pulverulent to floccose or floccose-fibrillose universal veil (q.v.), with such material mouse gray to light gray-brown (ca. 7C3), later decorated with light gray-brown (5B3 to 9.7YR 6.4/2.5) granules and light gray-brown to reddish brown fibrillose upward pointing scales, with such material evenly distributed or in narrow concentric bands, finely striatulate with some raised fibrils in undecorated areas below partial veil, with surface decoration becoming reddish brown from handling, cylindric or narrowing upward or broadest at mid-stipe or narrowest at mid-stipe, flaring at apex or not; *bulb* (30–) 57–112^{+} × 17–40 mm [shortest in mature specimen of Mueller 4368], napiform to narrowly napiform or dauciform, radicating, varying from poorly differentiated from stipe to subabrupt; *context* white to sordid white [bruising light yellow only in Mueller 4368], solid (under some conditions center of stipe becoming water soaked or gelatinized and pale sordid yellowish tan), sometimes with lacunae in center (especially in large specimens), infrequently becoming hollow [e.g., in Mueller 4368], concolorous to pale pallid brown or pale pinkish brown or ochraceous or pale brick-colored in larval tunnels; *partial veil* apical to superior, white becoming gray above, occasionally with edge darker gray in age, below bearing gray pulverulence, striate above, skirt-like, submembranous to floccose-felted, tearing, often left as floccose patches over lamellae; *universal veil* absent or as pyramidal warts or patches placed irregularly or subconcentrically arranged in few to many (up to 10 or more) rows near broadest point of bulb or for as much as upper three-quarters of bulb length, often with underlying context forming white to pale grayish recurved scales with universal veil remnants on tips of such scales, rather dark brownish gray (5.6YR 3.4/0.9), with limbus internus sometimes as thick, loosely attached, friable ring at stipe base (then concolorous with stipe).

Odor lacking or similar to nutmeg at first; in age, "old ham"-type, but more penetrating and unpleasant. ["Musty" in Mueller 4368.] *Taste* not distinctive.

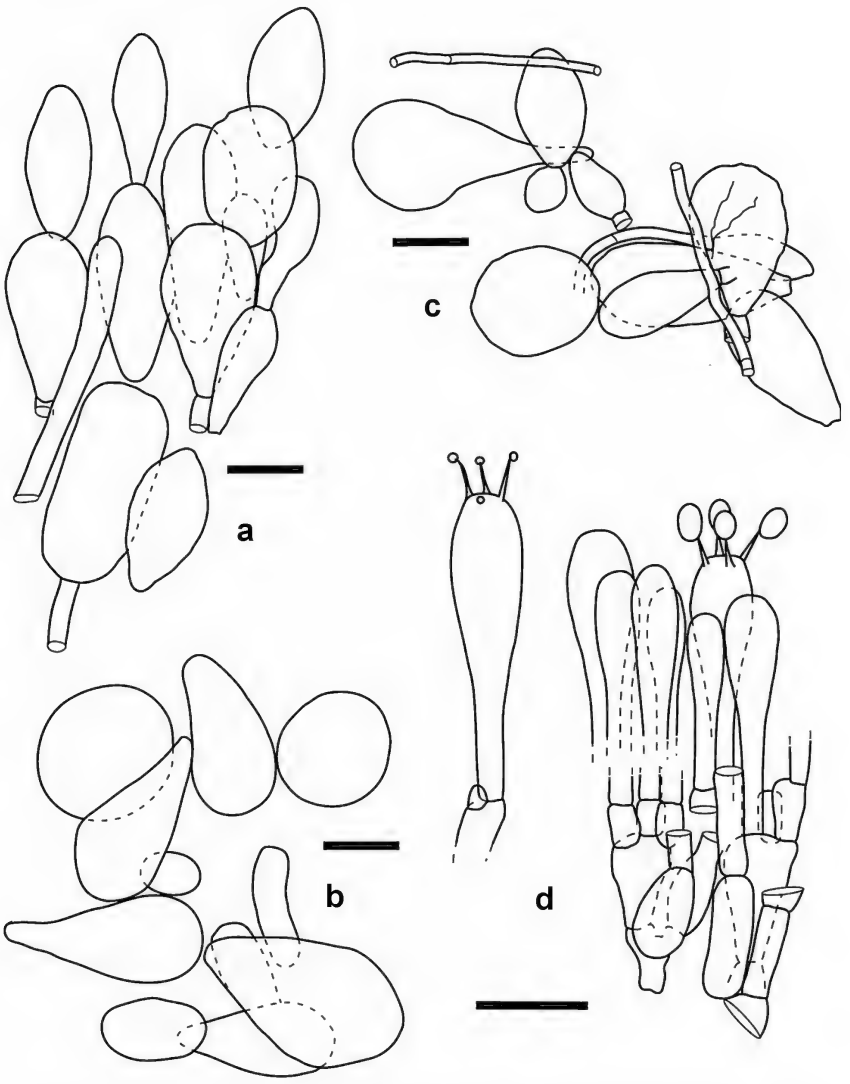

Fig. 11. *Amanita costaricensis*. a. Elements of universal veil from pileus wart (Tulloss 6-23-95-M). b. Elements of universal veil from floccose material of stipe (isotype). c. Elements of weakly structured partial veil (isotype). d. Elements of hymenium and subhymenial tree. Scale bars 20 µm.

MACROCHEMICAL TESTS: Spot test for laccase (syringaldazine): negative throughout basidiome except for very few spots on margins of lamellae. Spot

test for tyrosinase (paracresol): negative throughout basidiome. Test voucher: Tulloss 6-20-95-A.

PILEIPELLIS: 315–460 µm thick overall (175–235 µm in old specimen); suprapellis 95–230 µm thick (40–100 µm in old specimen), partially gelatinized, pale yellow to colorless except (occasionally) dark brownish orange at surface; subpellis 160–260 µm thick (130–135 µm in old specimen), ungelatinized, dark brownish orange; filamentous undifferentiated hyphae 2.8–8.4 µm wide, branching, densely packed, dominantly subradially oriented, but with some criss-crossing; vascular hyphae 7.4–21 µm wide, scattered to locally common, sinuous, with those of largest diameter from old (senescent?) specimen (Gómez & Alfaro 20641). PILEUS CONTEXT: filamentous undifferentiated hyphae 2.0–20 µm wide (many with width ca. 8.0 µm), branching, dominating, singly and in fascicles interwoven in open lattice, occasionally with yellowish subrefractive walls; inflated cells clavate to narrowly clavate to subfusiform, up to 138 × 34 µm, apparently mostly intercalary, one clavate cell terminal [possibly fitting definition of "acrophysalide" per Bas (1975)] except for hypha branching from one side, with walls thin or slightly thickened; vascular hyphae 8.4–31 µm wide, occasionally branching, sinuous, scattered to locally common especially just below pileipellis, locally in loose knots, with those of largest diameter from old (senescent?) specimen (Gómez & Alfaro 20641). LAMELLA TRAMA: bilateral, divergent; w_{cs} = 45–60± µm (moderate rehydration); central stratum composed largely of rather broad hyphae, apparently lacking intercalary inflated segments; subhymenial base comprising filamentous undifferentiated hyphae and narrow intercalary inflated cells (ellipsoid to obclavate to subfusiform to cylindric, up to 90 × 28 µm, singly and in short chains, with width predominantly 17.0–28 µm) diverging in smooth curve and achieving marked angle to central stratum; filamentous undifferentiated hyphae 2.8–10.2 µm wide, branching, sometimes constricted at septa; divergent, terminal inflated cells not observed; vascular hyphae not observed. SUBHYMENIUM: w_{st}-near = 65–85 µm (poor to moderate rehydration) or 145–150 µm (good rehydration); w_{st}-far = 85–110± µm (poor to moderate rehydration) or 170–175 µm (good rehydration); appearing pseudoparenchymatous in some regions, comprising 2–4 layers of inflated cells (sometimes dominating) and irregularly branched partially inflated to inflated elements and partially inflated (proportionately small) hyphal segments, with basidia arising from cells of all types. BASIDIA: (21–) 39–63 × (6.2–) 8.2–14.2 (–15.8) µm, thin-walled, 4-sterigmate, with sterigmata up to 6.0 × 2.8 µm or larger; clamps common. UNIVERSAL VEIL: *On pileus*: elements having strong vertical orientation except in base of wart; filamentous undifferentiated hyphae 1.0–13.6 µm wide, branching, common in upper part

of wart, dominating in shallow interwoven layer at base of wart and there periclinally oriented and more often in fascicles, sometimes constricted at septa, colorless at first becoming orange-brown like inflated cells; inflated cells pale brown to pale brownish gray to pale gray, becoming brownish orange to orange-brown to brown, 28–128 × 11.5–50 µm, dominating except in base of wart, terminal in short chains, thin-walled, subglobose to broadly ellipsoid to ellipsoid to broadly clavate (up to 83 × 50 µm) or basidiiform to narrowly clavate to subcylindric to cylindric to subfusiform (up to 128 × 37 µm), all forms occasionally rostrate when in terminal position in chain; vascular hyphae 7.2–10.2 µm wide sinuous, infrequent, scattered. *On mid-stipe region*: almost entirely comprising scattered inflated cells, ungelatinized to partially gelatinized to gelatinized, subglobose (e.g., 32 × 28 µm), ellipsoid (e.g., 38 × 26 µm), narrowly to broadly clavate (e.g., 56 × 33 µm), bacilliform (e.g., 32 × 8.6 µm). *On bulb of stipe*: comprising disordered elements, with greater proportion of filamentous undifferentiated hyphae than in universal veil on pileus, otherwise similar to remnants on pileus. STIPE CONTEXT: longitudinally acrophysalidic; filamentous undifferentiated hyphae 1.9–9.9 µm wide, plentiful, occasionally branching; acrophysalides plentiful, up to 406 × 33 µm; vascular hyphae 7.0–16.5 µm wide, infrequent, sinuous, yellowish, mostly near exterior surface. PARTIAL VEIL: with hyphae, elongate cells, and rows of cells sometimes subradially oriented; radially streaked on upper surface with partially gelatinized collapsed remains of lamella edge tissue; filamentous undifferentiated hyphae 1.5–7.5 µm wide, moderately common, branching occasionally, often single, sometimes in narrow fascicles, infrequently in fascicles up to 17.5 µm wide; inflated cells arising from nonvascular hyphae (see below for inflated cells arising from vascular hyphae) thin-walled, terminal, singly or in short chains (mostly of two), pyriform or ovoid or elongate or ellipsoid or subfusiform or subcylindric or broadly clavate or clavate or narrowly clavate, up to 113 × 29 µm, occasionally with yellowish walls, dominantly colorless, hyaline; vascular hyphae 2.8–5.0 µm wide, very infrequent, infrequently giving rise to yellow-walled terminal cell (elongate to ellipsoid, up to 26 × 22 µm). LAMELLA EDGE TISSUE: sterile.

BASIDIOSPORES: [321/16/10] (7.5–) 8.5–13.2 (–16.0) × (5.1–) 6.0–8.5 (–12.6) µm, (**L** = (9.1–) 9.4–12.3 µm; **L'** = 10.8 µm; **W** = 6.4–7.7 (–8.3) µm; **W'** = 7.2 µm; Q = (1.10–) 1.27–1.83 (–2.15); **Q** = 1.35–1.68 (–1.70); **Q'** = 1.51), hyaline, colorless, smooth, thin-walled, amyloid, acyanophilous, dominantly ellipsoid to elongate, infrequently broadly ellipsoid or cylindric, often at least somewhat adaxially flattened, occasionally expanded at one end; apiculus sublateral, small, cylindric; contents mono- to multiguttulate to granular; white in deposit.

ECOLOGY: Solitary to subgregarious. Costa Rica: At 1715–2500 m elev. In *Quercus-Magnolia* forest or in mixed *Quercus* forest sometimes with *Q. oocarpa* dominant. Honduras: At 1900± m elev. In cloud forest with *Quercus trichodonta* and other hardwoods.

MATERIAL EXAMINED: **COSTA RICA**: CARTAGO—San Cristobal, vii.1983 L.D. Gómez & R. Alfaro 21222 (F 1100824). GUANACASTE—Cacao no. 1, sendero a derrumbe, 3.vi.1996 C. Cano 197 (INBio; RET 325-4). SAN JOSÉ—La Chonta, v.1982 L.D. Gómez 18147 (F 1051869); Jaboncillo de Dota, 20.vi.1995 R.E. Halling & K. Shanks s.n. [Tulloss 6-20-95-A] (RET 330-3; USJ); El Jardín de Dota, vii.1983 L.D. Gómez & R. Alfaro 20641 (F 1073283); San Gerardo de Dota no. 1, 26.vii.1992 B. A. Strack, L.D. Gómez, G. Hewson & G.M. Mueller [Mueller 4398] (F 1102451), 21.vi.1995 R.E. Halling s.n. [Tulloss 6-21-95-M] (holotype, USJ; isotype, RET 330-4), 23.vi.1995 Thomas O'Dell s.n. [Tulloss 6-23-95-M] (RET 330-5; USJ); San Gerardo de Dota no. 4, 24.vii.1992 B.A. Strack, J. Polishook, L.D. Gómez, G. Hewson & G.M. Mueller [Mueller 4368] (F 1102421). **HONDURAS**: FRANCISCO MORAZAN—Mt. Uyuca, Valle Zamorano, 5.vii.1991 G.M. Mueller, B.A. Strack, R. & M. Singer & R. Andino [Mueller 4138] (F 1098682). OLANCHO—ca. Campamento, P.N. La Tigre, rd. to Finca Sapote, 9.vii.1991 G.M. Mueller, B.A. Strack, R. & M. Singer & R. Andino [Mueller 4182] (F 1098725).

COMMENTS — This species was described in the notebooks of the late Dr. Rolf Singer under his herbarium name "*costaricensis*." Since he originated the name and had a clear concept of its application and since we utilize collections annotated by him, we have included Dr. Singer as an author of the species.

According to the descriptions and keys of Bas (1969), *A. costaricensis* fits well within his stirps *Microlepis* of *Amanita* [sect. *Lepidella*] subsect. *Solitariae* Bas. Within this stirps, the present species is strikingly similar to *A. atkinsoniana* Coker (Coker 1917) and *A. onusta* (Howe) Sacc. (Saccardo 1891).

Amanita atkinsoniana (Tulloss 2011d) is moderately common in a range extending from southern Prov. Québec, Canada (Pomerleau 1980) to Michoacan edo., Mexico. *Amanita atkinsoniana* can be distinguished from the present species by

- smaller spores—[230/10/10] (6.8–) 8.0–10.5 (–14.3) × (5.0–) 5.4–7.2 (–8.5) µm, (**L** = (8.2–) 9.0–9.7 µm; **L'** = 9.2 µm; **W** = 5.7–6.6 (–6.9) µm; **W'** = 6.2 µm; Q = (1.17–) 1.27–1.71 (–1.95); **Q** = 1.36–1.57 (–1.67); **Q'** = 1.50)
- basidiome generally white or pallid except for universal veil, with universal veil in fine distinctly separated warts (grayish at first, then reddish brown) on pileus, with basal bulb usually napiform or turbinate, with universal veil brownish gray becoming reddish brown and distributed in rather fine warts in at least six (6) concentric rings around the stipe's bulb from its top to (often) well below mid-length of the bulb,

with fragile apical to subapical partial veil, which (when it persists) may become slimy and yellow while remaining in a superior position on the stipe

- scattered, minimal, positive spot tests for tyrosinase—with L-tyrosine, slowly positive on surfaces of lower stipe and upper bulb (possibly in association with universal veil material in those areas) and in pileipellis.

MATERIAL EXAMINED (*Amanita atkinsoniana*): **MÉXICO**: MICHOACAN—Parq. Nac. "Insurgente José Ma. Morelos," Mpio. Charo, 19.viii.1983 Reza Araujo s.n. (FCME). **U. S. A.**: CONNECTICUT—Middlesex Co. – E. Haddam, Devil's Hop Yard St. Pk., 25.ix.1999 Arnold s.n. [Tulloss 9-25-99-P] (RET 301-1); Meshomasic St. For., 19.ix.1998 Peter Kukle s.n. [Tulloss 9-19-98-A] (RET 288-2). MAINE—Cumberland Co. – S. Windham, 25.viii.1984 S.S. Ristich s.n. [Tulloss 8-25-84-SSR-A] (RET 235-6). MASSACHUSETTS—Berkshire Co. – Pittsfield St. For., 16.viii.1986 Wes Faust s.n. [Tulloss 8-16-86-G] (RET 137-7). NEW JERSEY—Morris Co. – Hacketstown Reservoir, 13.viii.1984 Robert Peabody & Roger Phillips s.n. [Tulloss 8-13-84-PPA] (RET 113-8). NEW YORK—Dutchess Co. – Thompson Pond Preserve, Pine Plains, 4.viii.1996 Wm. Bakaitis s.n. (NYS D1592). NORTH CAROLINA—Granville Co. – Butner, John Umstead Hospital picnic area, 18.xi.1994 Owen L. McConnell s.n. (RET 139-6). McDowell Co. – ca. Little Switzerland, Wildacres Resort, 27.ix.2008 J. Justice NC-AM12 (RET 445-1). Orange Co. – Chapel Hill, Battle's Pk., 14.ix.1913 W.C. Coker 759 (holotype, NCU, mixed collection segregated by C. Bas). WEST VIRGINIA—Greenbrier Co. – Monongahela Nat. For., Lake Sherwood, 31.viii.1982 R.E. Tulloss 8-31-82-D (RET 226-2). Tucker Co. – Dolly Sods, 1.viii.1985 W. Sturgeon s.n. [Tulloss 8-1-85-B] (RET 201-6).

Amanita onusta (Tulloss 2011e) is a locally common species of eastern North America with a range extending from Prov. Québec (Pomerleau 1980) and Prov. Nova Scotia (Stewart & Grund 1974), Canada at least to Illinois and Mississippi, USA. (Tulloss unpub. data). *Amanita onusta* can be distinguished from the present species by

- smaller spores—[215/12/12] (7.0–) 8.0–11.0 (–13.0) × (5.0–) 5.5–7.0 (–8.3) µm, (**L** = 8.3–10.5 (–11.0) µm; **L'** = 9.4 µm; **W** = 5.7–6.5 (–7.1) µm; **W'** = 6.1 µm; Q = (1.14–) 1.28–1.85 (–2.21); **Q** = 1.35–1.65 (–1.84); **Q'** = 1.52)
- basidiomes paler and with little (if any) brown tint, frequently of smaller size with pileus often less than 40 mm wide [13–76 mm wide in Tulloss' experience, although Bas (1969) reports at least one instance of a specimen with pileus over 100 mm wide], with lamellae that rather rapidly become yellowish and show signs of decay while the remainder of the basidiome shows none, and often without a persistent partial veil
- positive spot tests for tyrosinase—with L-tyrosine, positive, throughout the basidiome and, with paracresol, positive in scattered spots in very

young material, positive in pileus context and context of stipe above bulb in older material.

MATERIAL EXAMINED (*Amanita onusta*): **U. S. A.**: CONNECTICUT—Litchfield Co. – Black Rock St. Pk., 10.viii.1983 D.C. & R.E. Tulloss 8-10-83-A (RET 104-8). MAINE—Sagadahoc Co. – Woolwich, 7.x.1995 S.S. Ristich s.n. (RET 159-7). NEW JERSEY—Mercer Co. – Hightstown, 22.vii.1981 R.E. Tulloss 7-22-81-B (RET 106-9). Monmouth Co. – Upper Freehold Twp., Assunpink Wildlife Mgt. Area, Roosevelt Rd., 21.vii.1981 R.E. Tulloss 7-21-81-E (RET 163-3), 4.viii.1981 R.E. Tulloss 8-4-81-A (RET 109-6), 18.ix.1981 M.A. King & R.E. Tulloss 9-18-81-E (RET 164-7), 11.vii.1982 D.C., M.H., & R.E. Tulloss 7-11-82-B (RET 340-10). Morris Co. – Morristown, 27.viii.1981 Al Northrup s.n. [Tulloss 8-27-81-AN] (RET 166-10). VIRGINIA—Franklin Co. – ca. Ferrum, ca. Ferrum College, 4.viii.1982 Gerald Bills, Pierre Dery, O.K. & H.H. Miller OKM19946 (VPI, as "*A. peckiana*"). WEST VIRGINIA—Marion Co. – Mill Fall Run, 21.ix.1992 R.P. Bhatt A2 (FWVA).

In two cases [Halling 7667 (NY; USJ) and Tulloss 6-21-95-E (RET 337-3; USJ)], an amanita assignable to *Amanita* subgenus *Lepidella* (sect. *Validae* or sect. *Lepidella*) was misdetermined in the field as *A. costaricensis*. The undetermined species has a gray stipe that is stuffed (becoming hollow), gray underside and margin to the membranous annulus, gray warts on the gray-brown to pale gray pileus, and short basidia [19–34 (–44) µm long] apparently lacking clamps. The species is somewhat unusual for sect. *Validae* because of its unpleasant smell (sometimes like the smell of disinfectant cleaning solution in hospitals). The misdetermined entity should be distinguishable by the lack of a radicating bulb, the absence of basidial clamps, and by its smaller and more nearly globose spores: [33/2/2] (7.3–) 7.5–9.0 (–10.0) × (6.5–) 7.0–8.0 (–9.1) µm, (**L** = 8.2 µm; **L'** = 8.2 µm; **W** = 7.5 µm; **W'** = 7.5 µm; Q = (1.04–) 1.05–1.14 (–1.23); **Q** = 1.09–1.11; **Q'** = 1.09). Especially, considering the apparently parasitized collections of *A. costaricensis* discussed below, we had some hesitation concerning whether or not Halling 7667 and Tulloss 6-21-95-E also comprised parasitized material. However, we are convinced that they represent a distinct species because (1) the spores of these two collections were both shorter (on average) and broader (on average) than those of *A. costaricensis* [Compare values of **L'** and **W'**. Reduction in spore volume due to stress would have reduced both length and width.]; (2) both exhibited a membranous annulus; and (3) both apparently lack basidial clamps. Basidia predominantly less than 30 µm long, might support assignment to sect. *Validae*, but further study is required.

In one case {Estrella, 14.vi.1996 R.E. Halling & J. Ammirati [Halling 7684] (NY; USJ)}, all the specimens of a rather large collection apparently assignable to *A. costaricensis* had an odor like pig manure. In addition, some had rather distorted stipes; and the spores of all collections were uniformly smaller

than normal ([100/5/1] (7.2–) 7.6–9.5 (–11.0) × (5.3–) 5.5–7.3 (–8.0) µm, (**L** = 8.3–9.0 µm; **L'** = 8.7 µm; **W** = 6.2–6.6 µm; **W'** = 6.4 µm; Q = (1.18–) 1.23–1.50 (–1.80); **Q** = 1.30–1.45; **Q'** = 1.37). Observed at 1250×, many of the spores were surrounded with small, hyaline, colorless, inamyloid spheres (1±–3± µm diam.) connected to the spore walls by what appeared to be fine tubules—the whole having the appearance of a pincushion. We hypothesize that the noted alterations in morphology and odor are a result of "parasitization." The authors explicitly exclude Halling 7684 from the set of paratypes.

In another case of apparent parasitization (San Gerardo de Dota no. 4, 24.vii.1992 B.A. Strack, J. Polishook, L.D. Gómez, G. Hewson & G.M. Mueller [Mueller 4368] (F 1102421)), spores, basidia, and lamella trama were quite normal for the species; however, the context of both the pileus and stipe stained yellow when cut. Two other pigment-related characters were unusual for *A. costaricensis* as we understand it: universal veil remnants on pileus, stipe, and bulb of stipe were paler than usual and remained paler than the pileus disc despite becoming brown with time, even in a mature specimen; and the pileus disc was darker than usual. Further, lamellulae were reported to all be very short; and the basidiomes were said to have had an unusual musty odor. The yellow staining is very similar to that seen in apparently parasitized specimens of *A. subsolitaria* (Murrill) Murrill from the Atlantic coastal plain of the USA (Tulloss 1998b; 2000a: 57, fig. 5; Tulloss 2011c). In the latter case, basidiomes are sometimes found that not only stain bright yellow when cut, but release an orange-amber liquid from wounds. Spores of such specimens are often, but not always, subnormal in size and distorted in shape—the same yellowing phenomenon may be seen in specimens with spores nearly, or quite, normal with regard to size and shape. The provisional name of Bas (1969), *A. "crassifolia,"* was put forward for yellowing material that Bas thought might be determinable as *A. subsolitaria*; Tulloss is now convinced this is the case.

It should be noted that two North American taxa assignable to sect. *Lepidella* have been described by Bas (1969) because of their pronounced ability to become bright yellow or yellow-orange when cut (*A. cinereoconia* var. *croceescens* Bas and *A. rhoadsii* var. *flavotingens* Bas). Tulloss (1998b, 2000a) questioned whether these entities might not be based on parasitized specimens of the respective species. It would be interesting to see what information about *Amanita* parasites might be determined from study of fresh material of Costa Rican cases of apparent parasitization such as those presented above. If the species with yellowing context reaction in sect. *Lepidella* may be grouped together and the yellowing attributed to a single cause, or a set of common causes, then *A. costaricensis* becomes the first representative of this group in

Bas' stirps *Microlepis*. We have included Mueller 4368 as a paratype because microscopic examination produced observations completely consistent with "normal" material of *A. costaricensis*. In the macroscopic description of *A. costaricensis*, the unusual characters of Mueller 4368 are so labeled.

Mueller 4398 consists of a single immature specimen with the partial veil covering the lamellae.

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Appendix: Gazetteer of collecting sites in Costa Rica referenced in this and planned future studies on *Amanita*

Localities are designated by uncomplicated names in the taxonomic part of this paper. Full site descriptions with latitude, longitude, elevation, and the species of *Quercus* present at the site (where data is available) are provided in the following table. Full data was sometimes not available because of the difficulty of interpreting herbarium labels on collections made prior to the current projects. Most of our data comes from the latter. Also, milestones along the Interamerican Hwy. have been moved over time, and the mention of a milestone in an old locality description may be less accurate than the apparently less precise or more informal part of the description. The issue of incompletely documented sites arises largely because of the number of good quality "historic" collections in F. Latitude, longitude, and elevation for recently explored sites were determined by use of a Garmin (GPS75) device.

Site Name	Site Description (including Oaks Present)	Lat./Long., Elevation
PROV. ALAJUELA		
1. Bosque del Niño	Ctn. Grecia, Grecia, Bosque del Niño - *Q. seemannii* (dominant)	10°9′4″N/84°14′42″W, 1900 m elev.
2. Palmira	? - no data on oaks	ca. 10°12′N/84°23′W, no data on elev.
PROV. CARTAGO		
3. Cañon	ca. Cañon, E of Interam. Hwy., at La Esperanza del Guarco - *Q. copeyensis* (dominant)	9°41′1″N/83°52′43″W, 2505 m elev.
4. Estrella	5 km E of km 31 of Interam. Hwy., ca. Estrella - *Q. oocarpa, Q. seemannii*	9°46′4″N/83°57′19″W, 1685-1717 m elev.
5. Palo Verde	4± km E of km 31 of Interam. Hwy., ca. Palo Verde - *Q. oocarpa*	9°46′34″N/83°56′42″W, 1600 m elev.
6. [unnamed]	off Interamerican Hwy., 6 km S of Cartago at rd. to Palo Verde - no data on oaks	?, ? m elev.
7. Prusia	Parque Prusia, W slope of Volcán Irazu - *Q. costaricensis*	9°57′56″N/83°52′15″W, 2900 m elev.
8. San Cristobal	unknown site along Interam. Hwy. - no data on oaks	?, 2000 m elev.
9. Sanatorio	? - no data on oaks	?, ? m elev.

Site Name	Site Description (including Oaks Present)	Lat./Long., Elevation
10. Tapanti	Guarco, Tapanti, P. N. Tapanti, Macizo de la Muerte, Área de Conservación La Amistad Pacifico - *Q. copeyensis*	9°4′6″N/83°52′30″W, 2600 m elev.
PROV. GUANACASTE		
11. Cacao no. 1	Cerro Pedregal, Estación Biología Cacao, Área de Conservación Guanacaste - *Q. brenesii*	10°55′45″N/85°28′37″W, 1100 m elev.
12. Cacao no. 2	Cerro Cacao, Estación Biología Cacao, Área de Conservación Guanacaste - *Q. brenesii*	10°56′8″N/85°27′14″W, 1000 m elev.
PROV. HEREDIA		
13. San José de la Montaña	Volcán Barva, Paso Llano, San José de la Montaña - no data on oaks	ca. 10°3′N/84°7′W, 2100 m elev.
PROV. PUNTARENAS		
14. Finca Las Alturas	Ctn. Coto Brus, Finca Las Alturas, Standford Biol. Field Stn., tr. to Cerro Echandi - no data on oaks	?, 1700 m elev.
15. La Amistad no. 1	Ctn. Coto Brus, La Amistad, Zona Protectora Las Tablas, Finca La Cafrosa, Camino El Portones por El Tajo - *Q. corrugata* & *Q. seemannii*	8°55′34″N/82°46′0″W, 1350–1500 m elev.
16. La Amistad no. 2	Ctn. Coto Brus, Las Mellizas, La Amistad Lodge, ca. P. N. La Amistad - *Q. corrugata* & *Q. seemannii*	8°54′52″N/82°46′50″W, 1380 m elev.
17. San Vito	Ctn. Coto Brus, San Vito de Coto Brus, 5 km W of Jardín Botánico Las Cruces - no data on oaks	ca. 8°49′N/83°0′W, 1500 m elev.
PROV. SAN JOSÉ		
18. Alto de la Palma	Ctn. Guadalupe, Alto de la Palma, ? - no data on oaks	?, ? m elev.
19. C.A.T.I.E. For.	Ctn. Perez Zeledón, Villa Mills, C.A.T.I.E. Exp. For. of Villa Mills - *Q. costaricensis*	9°33′3″N/83°40′55″W, 2880 m elev.

Site Name	Site Description (including Oaks Present)	Lat./Long., Elevation
20. Copey de Dota	Ctn. Dota, Copey de Dota 12 km S of Copey on rd. to Providencia - *Q. copeyensis*	9°35′19″N/83°53′3″W, 2800 m elev.
21. El Empalme	Ctn. Dota, 200± m W of Interam. Hwy. at El Empalme, La Guaria [Note: Site has been completely logged.] - *Q. copeyensis*	9°43′8″N/83°57′4″W, 2250 m elev.
22. Finca Alejandrina	Ctn. Perez Zeledón, Villa Mills, Finca Alejandrina, Interam. Hwy. km 95, ca. Hotel La Georgina - *Q. costaricensis*	9°33′43″N/83°44′22″W, 3000 m elev.
23. Jaboncillo de Dota	Ctn. Dota, Jaboncillo de Dota, 3.2 km from Interam. Hwy on rd. to San Gerardo de Dota - *Q. copeyensis*	9°35′21″N/83°47′58″W, 2740 m elev.
24. Jardín de Dota	Ctn. Dota, 3.5 km W of Interam. Hwy. at El Empalme - *Q. copeyensis*, *Q. seemannii* (occas.)	9°42′52″N/83°58′28″W, 2220 m elev.
25. La Chonta	Ctn. Dota, S of Interam. Hwy. twd. Cerro Chonta -*Q. copeyensis*, *Q. seemannii*, *Q. rapurahuensis*	9°41′58″N/83°56′31″W, 2400 m elev.
26. San Gerardo de Dota no. 1	Ctn. Dota, San Gerardo de Dota, 5± km SW of Cerro de la Muerte, Albergue de Montaña Savegre (Cabinas Chacón) - *Q. copeyensis*, *Q. seemannii*, *Q. rapurahuensis*	9°33′2″N/83°48′27″W, 2200 to *ca.* 2350 m elev.
27. San Gerardo de Dota no. 2	Ctn. Dota, 500 m from Interam. Hwy. on rd. to San Gerardo de Dota - *Q. costaricensis*	9°36′13″N/83°47′26″W, 3000 m elev.
28. San Gerardo de Dota no. 3	Ctn. Dota, 1.5 km from Interam. Hwy. on rd. to San. Gerardo de Dota - *Q. copeyensis*, *Q. costaricensis*	9°35′47″N/83°47′55″W, 2860 m elev.
29. San Gerardo de Dota no. 4	Ctn. Dota, off Interam. Hwy., 2–3 km S of Cerro de la Muerte, rd. to San Gerardo de Dota - no field data on oaks; *Q. copeyensis* and *Q. rapurahuensis* likely, *Q. seemannii* possible	?, btwn. 2200 to 2860 m elev.

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Studies on *Cephalotrichum* from soils in China — twelve new species and two new combinations

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Abstract — A study of *Cephalotrichum* species diversity in soils of China resulted in twelve new species: *Cephalotrichum acutisporum*, *C. cylindrosporum*, *C. ellipsoideum*, *C. inflatum*, *C. longicollum*, *C. macrosporum*, *C. oblongum*, *C. ovoideum*, *C. robustum*, *C. spirale*, *C. terricola*, *C. verrucipes*. These species are illustrated and described in this paper. The type specimens (dried cultures) and living cultures are deposited in the Plant Pathology Herbarium of Shandong Agricultural University (HSAUP). Two *Doratomyces* species are transferred to *Cephalotrichum*.

Key words — anamorphic fungi, taxonomy

Introduction

The genus *Cephalotrichum* was established by Link (1809) with *C. stemonitis* as type species. This genus produces its spores in a dry head at the apex of a complex, erect conidiophore or synnema, which is often up to a millimeter in height. The synnemata produce chains of powdery conidia with a 'bottle brush' or 'feather' appearance. The ovoid conidia are produced from annellidic conidiogenous cells covering the sporogenous area (Chlebicki 2008). About 50 taxa of *Cephalotrichum* have been reported (http://www.indexfungorum.org/Names/Names.asp).

Doratomyces was proposed by Corda (1829), with *D. neesii* as type species. However, the probable synonymy of *Cephalotrichum* and *Doratomyces* has been a subject of debate for many years. Hughes (1958) and von Arx (1981) accepted *Doratomyces* as a synonym of *Cephalotrichum*, whereas Morton & Smith (1963),

Y.L. Jiang and J.J. Xu contributed equally to this work.

Domsch et al. (2007), and Kirk et al. (2008) accepted *Doratomyces* as a separate genus. In this paper, we treat *Doratomyces* as a synonym of *Cephalotrichum*.

During research on soil dematiaceous hyphomycetes in China, we obtained twelve fungi that possessed the typical *Cephalotrichum* characters but did not match other species in this genus. These fungi are described as new taxa and two *Doratomyces* species are transferred to *Cephalotrichum*, as new combinations.

Materials & methods

The methods of sample collection, isolation and identification of fungi, and preparation of dried cultures were as previously reported by Jiang & Zhang (2007). Conidia and conidiophores were placed in a drop of lactic acid, examined, and photographed using light microscopy. Mature conidia and conidiophores were measured by using a Nikon 90i microscope (Nikon Corporation, Japan) at 100× magnification. The fungi are described from cultures grown on potato dextrose agar (PDA; 20 g white potato boiled and filtered, 20 g dextrose, 20 g agar, 1 L distilled water).

Taxonomy

Cephalotrichum acutisporum J.J. Xu & T.Y. Zhang, **sp. nov.** FIG. 1

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Coloniae effusae, floccosae vel pulveraceae, fuscae vel atrae, in PDA crescentes lentissime et attingentes diametrum 2–4 centimetri ad 25°C post duas hebdomadas, reverse atro-brunneae vel fuscae. Mycelium plerumque superficiale, ex hyphis pallide brunneis vel brunneis, laevibus, septatis, ramosis, 1.5–3.5 µm crassis, in funiculos floccosos interdum assurgens. Synnemata 120–820 µm alta, stipitata, atro-brunnea vel fusca, capitulo cylindrica et ellipsoidea. Conidiophora pallide brunnea vel brunnea, ramosa, septata, laevia, 1–4 µm lata, basin 1.5–3.5 µm lata. Celluae conidiogenae percurrentes (annellophora), ampuliformes, penicilliformiter dispositae, 4–10 × 2–3 µm. Conidia catenata, ellipsoidea vel cylindrica, basin truncata et apicem acuta, dilute brunnea vel brunnea, laevia, 5–6 × 2.5–3 µm.

Holotype: China. Fujian Province, Zhangping, from soil of a park, 22 October 2004, J.J. Xu (HSAUPII$_{04}$2724, dried culture, **holotype**; HMAS196222, **isotype**).

Etymology: in reference to the conidia, which are pointed at the apex.

Colonies on PDA effuse, floccose or powdery, at first greenish grey, finally blackish brown to black with the formation of abundantly sporing structures and synnemata, reverse dark brown to blackish brown, growing slow, reaching 2–4 cm diameter at 25°C after two weeks. Mycelium mostly superficial, composed of pale to mid brown, smooth, septate, branched, 1.5–3.5 µm wide hyphae that occasionally aggregate forming ropes. Synnemata 120–820 µm high, stipes dark brown to blackish brown, with cylindrical or ellipsoidal heads. Conidiophores pale brown to brown, branched, septate, smooth, 1–4 µm wide, 1.5–3.5 µm wide at the base. Conidiogenous cells (annellophores) ampuliform, penicillately arranged, 4–10 × 2–3 µm. Conidia catenate, ellipsoidal to cylindrical, truncate at the base and pointed at the apex, pale to mid brown, smooth, 5–6 × 2.5–3 µm, L/W = 2.

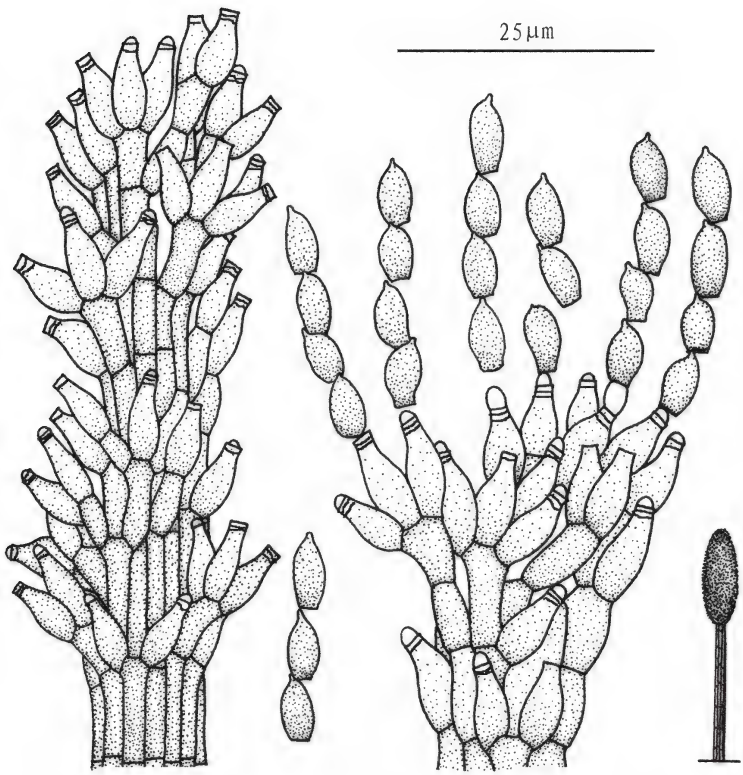

Fig. 1. *Cephalotrichum acutisporum*: conidia, conidiogenous cells and synnema.

Comments: *C. acutisporum* is most similar to *C. stemonitis* (Pers.) Link (Link 1809) and *C. microsporum* (Sacc.) P.M. Kirk (Kirk & Spooner 1984). However, conidia of *C. acutisporum* are ellipsoidal to cylindrical, but those of *C. microsporum* and *C. stemonitis* are ovoid. Conidia are larger (6–8.5 × 4–4.5 µm) in *C. stemonitis* and broader (3–4 µm) in *C. microsporum* than those of *C. acutisporum,* while synnemata are shorter (600 µm) in *C. microsporum* and longer (≤ 1200 µm) in *C. stemonitis* (Ellis 1971, Morton & Smith 1963).

Cephalotrichum cylindrosporum Y.L. Zhang & T.Y. Zhang, **sp. nov.** Fig. 2

MycoBank MB 561114

Coloniae effusae, pulveraceae, glaucae vel nigro-virentes, reverse brunneae, in PDA crescentes lentissime et attingentes diametrum 3–4 centimetri ad 25°C post duas hebdomadas. Mycelium plerumque superficiale, ex hyphis dilute brunneis vel brunneis, laevibus, septatis, ramosis, 2–2.5 µm crassis. Synnemata 750–900 µm alta, capitulo

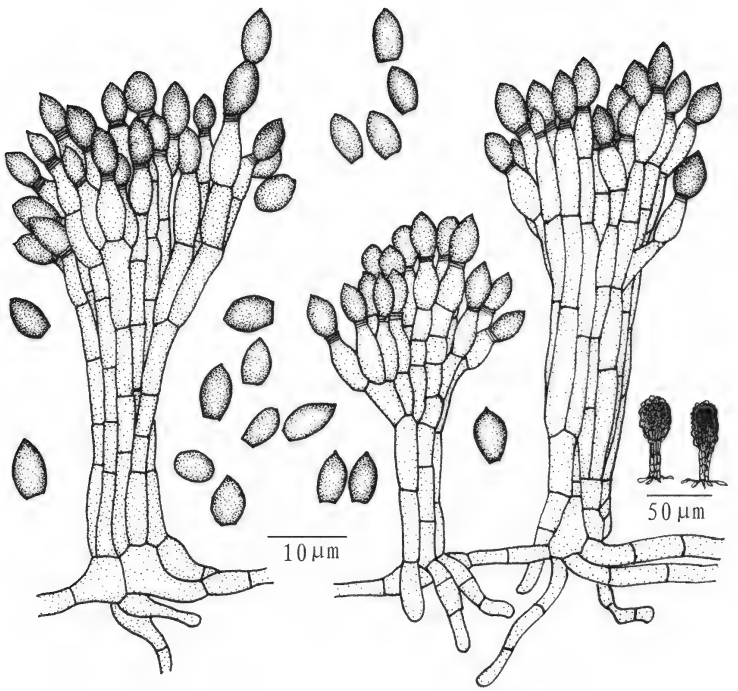

FIG. 2. *Cephalotrichum cylindrosporum*: conidia, conidiogenous cells and synnemata.

elongato-fusiformi stipitata, atro-brunnea, 15–20 μm lata. Celluae conidiogenae percurrentes (annellophora), ampuliformes, penicilliformiter dispositae, pallide brunneae vel brunneae, laeves, 9–11.5 × 2.5–3.5 μm. Conidia acrogena, solitaria vel catenata, cylindrica vel subcylindrica, in extremitatibus rotundata, brunnea, laevia, 5.0–6.2 × 2.5–3.2 μm.

HOLOTYPE: China. Hainan Province, Tunchang, from a rice field soil, 1 November 2005, Y.L. Zhang (HSAUPII$_{05}$2414, dried culture, **holotype**; HMAS196223, **isotype**).

ETYMOLOGY: in reference to cylindrical conidia.

Colonies on PDA effuse, powdery, glaucous to greenish black with the formation of abundantly sporulating structures and synnemata, reverse brown, growing slow, reaching 3–4 cm diameter at 25°C after two weeks. Mycelium mostly superficial, composed of pale brown to brown, smooth, septate, branched, 2–2.5 μm wide hyphae. Synnemata 750–900 μm high, with long fusiform heads, stipes dark brown, 15–20 μm wide. Conidiogenous cells (annellophores) ampuliform, penicillately arranged, pale brown to brown, smooth, 9–11.5 × 2.5–3.5 μm. Conidia acrogenous, simple or occasionally catenate, cylindrical or subcylindrical, rounded at the ends, brown, smooth, 5.0–6.2 × 2.5–3.2 μm.

COMMENTS: *Cephalotrichum cylindrosporum* is similar to *C. cuneiferum* in the shape of conidia, but in *C. cuneiferum* the conidia are longer (6–13 × 2.5–4 μm), synnemata are generally wider (10–40 μm), and the conidiogenous cells are longer (10–35 μm) (Matsushima 1975).

Cephalotrichum ellipsoideum H.Q. Pan & T.Y. Zhang, **sp. nov.** FIG. 3

MYCOBANK MB 561108

Coloniae effusae, pulveraceae, griseo-brunnea, reverse brunnea, in PDA crescentes lentissime et attingentes diametrum 2.5–4 centimetri ad 25°C post duas hebdomadas. Mycelium plerumque superficiale, ex hyphis dilute brunneis vel brunneis, laevibus, septatis, ramosis, 1.5–2.5 μm crassis, in funiculos floccosos saepe assurgens. Synnemata 100–500 μm alta, capitulo ellipsoidea et ovoidea, stipitata, atro-brunnea, 6–14 μm lata. Conidiophora brunnea, septata, ramosa. Celluae conidiogenae percurrentes (annellophora), ampuliformes, penicilliformiter dispositae, 7.5–12.5 μm longae, basi tumidulae 3–4 μm diam, quae in zonam annellatam, 1.5–3 μm diam, dilute brunneae, laeves. Conidia catenata, late ellipsoidea vel obovoidea, basin truncata et apicem rotundata, brunnea, laevia, 6–8.5 × 3.5–6 μm.

HOLOTYPE: China. Qinghai Province, Maduo, from a grassland soil, 8 June 2007, H.Q. Pan (HSAUPII$_{07}$4053, dried culture, **holotype**; HMAS196224, **isotype**).

ETYMOLOGY: in reference to the ellipsoidal conidia.

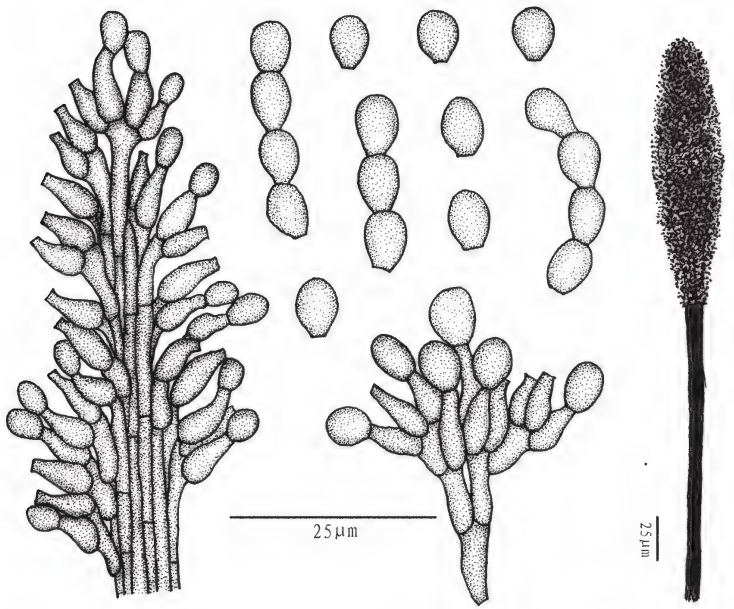

FIG. 3. *Cephalotrichum ellipsoideum*: conidia, conidiogenous cells and synnema.

Colonies on PDA effuse, powdery, greyish brown, reverse brown, growing slow, reaching 2.5–4 cm diameter at 25°C after two weeks. Mycelium mostly superficial, composed of pale brown to brown, smooth, septate, branched, 1.5–2.5 µm wide hyphae which usually aggregate forming ropes. Synnemata 100–500 µm high, with ellipsoidal to ovoid heads, stipes dark brown, 6–14 µm wide. Conidiophores brown, septate, branched. Conidiogenous cells (annellophores) ampuliform, penicillately arranged, 7.5–12.5 µm long, swollen base 3–4 µm diam, tapering abruptly into the annellated zone 1.5–3 µm diam, pale brown, smooth. Conidia catenate, broadly ellipsoidal to obovoid, truncate at the base and rounded at the apex, brown, smooth, 6–8.5 × 3.5–6 µm.

Comments: The conidia of *C. ellipsoideum* resemble those of *C. purpureofuscum* (Schwein.) S. Hughes (Hughes 1958), which, however, are slightly smaller (5–7 × 3.5–4.5 µm). In *C. purpureofuscum* the synnemata are also differently shaped (globose or subglobose) and taller (≤ 900 µm) (Ellis 1971).

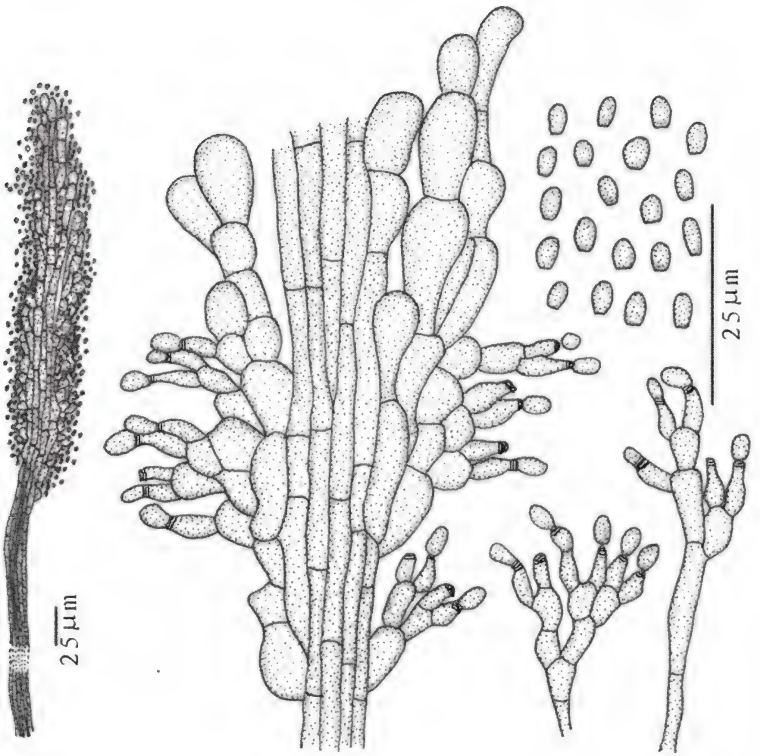

Fig. 4. *Cephalotrichum inflatum*: conidia, conidiogenous cells and synnema.

Cephalotrichum inflatum Y.L. Jiang & T.Y. Zhang, **sp. nov.** Fig. 4

MycoBank MB 561110

Coloniae effusae, floccosae, griseo-brunneae, reverse brunneae. Mycelium partem superficiale et partem immersum, ex hyphis hyalinis, ramosis, septatis, laevibus, 1.5–5.5 µm crassis. Synnemata 160–700 µm alta, capitulo fusiformia et subcylindrica, stipitata, atro-brunnea, 9–30 µm lata. Conidiophora inflata. Celluae conidiogenae percurrentes (annellophora), ampuliformes, penicilliformiter dispositae, 3–6.5 µm longae, basi tumidulae 2–3 µm diam, quae in zonam annellatam, 1–2 µm diam. Conidia solitaria vel catenata, late obovoidea vel subellipsoidea, basin truncata, apicem rotundata vel leviter acuta, laevia, pallide brunnea vel brunnea, 3–4.5 × 2–2.5 µm.

Holotype: China. Sichuan Province, Mianyang, from a mountain soil, 8 August 2005, Y.L. Jiang (HSAUPII$_{05}$0918, dried culture, **holotype**; HMAS196226, **isotype**).

Etymology: in reference to the inflated conidiophores.

Colonies on PDA effuse, at first powdery, finally floccose, greyish brown, reverse brown. Mycelium partly superficial, partly immersed, composed of hyaline, branched, septate, smooth, 1.5–5.5 µm wide hyphae. Synnemata 160–700 µm high, with fusiform or subcylindrical heads, stipes dark brown, 9–30 µm wide. Conidiophores often inflated. Conidiogenous cells (annellophores) ampuliform, penicillately arranged, 3–6.5 µm long, swollen base 2–3 µm diam, tapering abruptly into the annellated zone 1–2 µm diam. Conidia solitary or occasionally catenate, broadly obovoid to subellipsoidal, truncate at the base, rounded or slightly pointed at the apex, smooth, pale to mid brown, 3–4.5 × 2–2.5 µm.

Comments: *Cephalotrichum inflatum* differs from all previously described *Cephalotrichum* species by its distinctly inflated conidiophores.

Cephalotrichum longicollum Y.L. Jiang & T.Y. Zhang, **sp. nov.** Fig. 5

MycoBank MB 561111

Coloniae effusae, floccosae, fuscae, reverse brunneo-virens. Mycelium partem superficiale et partem immersum, ex hyphis hyalinis, ramosis, septatis, laevibus, 1–3 µm crassis. Synnemata 340–750 µm alta, capitulo elongato-cylindrica, stipitata, atro-brunnea, 6–25 µm lata. Celluae conidiogenae percurrentes (annellophora) ampuliformes, longicollo, penicilliformiter dispositae, 9–15.5 µm longae, basi tumidulae 3–6 µm diam, quae in zonam annellatam, 1–2.5 µm diam. Conidia solitaria vel catenata, ellipsoidea vel late ellipsoidea, brunnea, basin truncata, apicem rotundata vel interdum leviter acuta, laevia, 4.5–5.5 × 2.5–4 µm.

Holotype: China. Sichuan Province, from a soil of Emei Mountain, 9 August 2005, Y.L. Jiang (HSAUPII$_{05}$0802, dried culture, **holotype**; HMAS196228, **isotype**).

Etymology: in reference to the annellophores with long necks.

Colonies on PDA effuse, at first powdery, finally floccose, blackish brown, reverse greenish brown. Mycelium partly superficial, partly immersed, composed of hyaline, branched, septate, smooth, 1–3 µm wide hyphae. Synnemata 340–750 µm high, with long cylindrical heads, stipes dark brown, 6–25 µm wide.

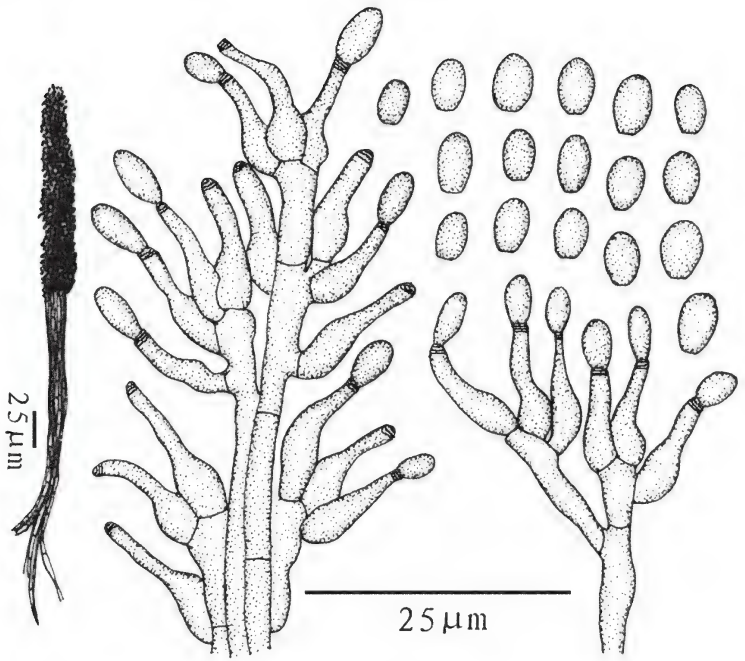

Fig. 5. *Cephalotrichum longicollum*: conidia, conidiogenous cells and synnema.

Conidiogenous cells (annellophores) ampuliform with long neck, penicillately arranged, 9–15.5 µm long, swollen base 3–6 µm diam, tapering abruptly into the annellated zone 1–2.5 µm diam. Conidia solitary or catenate, ellipsoidal to broadly ellipsoidal, smooth, mid brown, truncate at the base, rounded or sometimes slightly pointed at the apex, 4.5–5.5 × 2.5–4 µm.

Comments: *Cephalotrichum longicollum* is distinguished from all previously described *Cephalotrichum* species by annellophores with long neck and wider (3–6 µm) swollen base. Only *C. cuneiferum* (Berk. & Broome) Kuntze (Kuntze 1898) produces annellophores with a longer neck. However, shape of the conidia distinguishes these two species (Matsushima 1975).

Cephalotrichum macrosporum Y.L. Jiang & T.Y. Zhang, **sp. nov.** Fig. 6

MycoBank MB 561112

Coloniae effusae, floccosae, griseo-brunneae, reverse fuscae. Mycelium partem superficiale et partem immersum, ex hyphis hyalinis, ramosis, septatis, laevibus, 1–3 µm crassis. Synnemata 200–600 µm alta, capitulo cylindrica vel elongato-cylindrica, stipitata, atro-brunnea, 10–22 µm lata. Celluae conidiogenae percurrentes (annellophora), ampuliformes, penicilliformiter dispositae, 6.5–13 µm longae, basi tumidulae 2.5–3.5 µm diam, quae in zonam annellatam, 1–2.5 µm diam. Conidia solitaria vel catenata, cylindrica vel ovoidea,

longa ellipsoidea, brunnea, laevia, basin truncata, apicem rotundata, 5.5–12 × 2.5–4.5 µm.

Holotype: China. Sichuan Province, from a forest soil of Jiuzhaigou, 18 August 2005, Y.L. Jiang (HSAUPII$_{05}$0878, dried culture, **holotype**; HMAS196229, **isotype**).

Etymology: in reference to the large conidia.

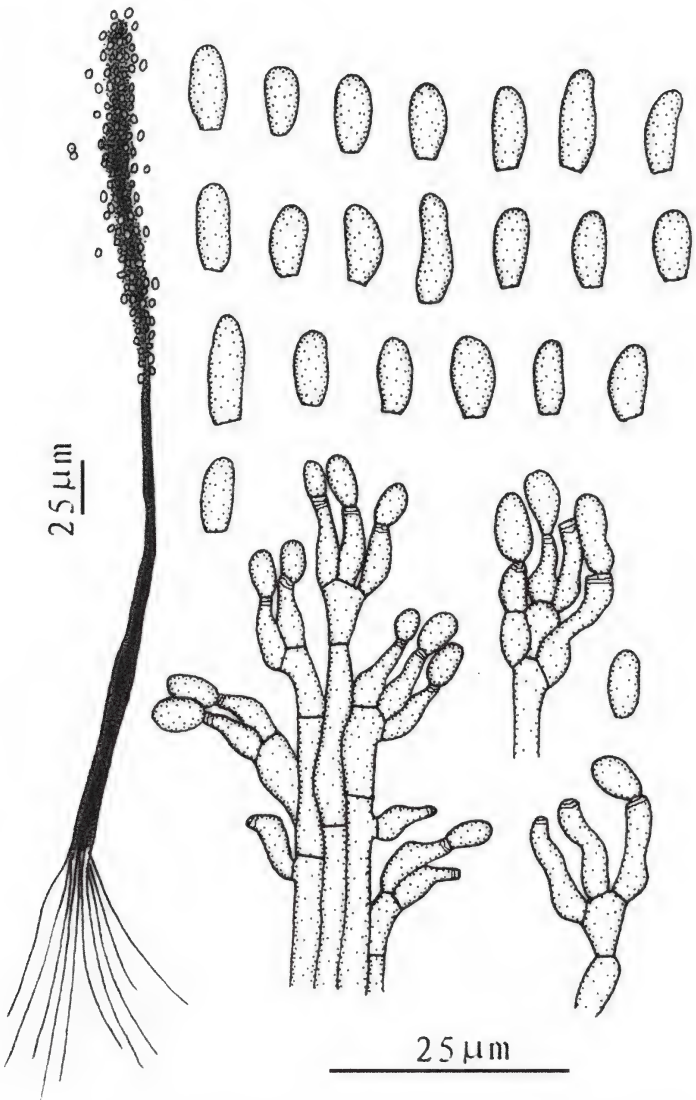

Fig. 6. *Cephalotrichum macrosporum*: conidia, conidiogenous cells and synnema.

Colonies on PDA effuse, at first powdery, finally floccose, greyish brown, reverse blackish brown. Mycelium partly superficial, partly immersed, composed of hyaline, branched, septate, smooth, 1–3 µm wide hyphae. Synnemata 200–600 µm high, with cylindrical to long cylindrical heads, stipes dark brown, 10–22 µm wide. Conidiogenous cells (annellophores) ampuliform, penicillately arranged, 6.5–13 µm long, swollen base 2.5–3.5 µm diam, tapering abruptly into the annellated zone 1–2.5 µm diam. Conidia solitary or catenate, cylindrical to ovoid or long ellipsoidal, mid brown, smooth, truncate at the base and rounded at the apex, 5.5–12 × 2.5–4.5 µm.

Comments: *Cephalotrichum cuneiferum* also produces cylindrical conidia but they are slightly larger than those of *C. macrosporum*. In addition, both the annellophores and synnemata of *C. macrosporum* are shorter than *C. cuneiferum* (Matsushima 1975).

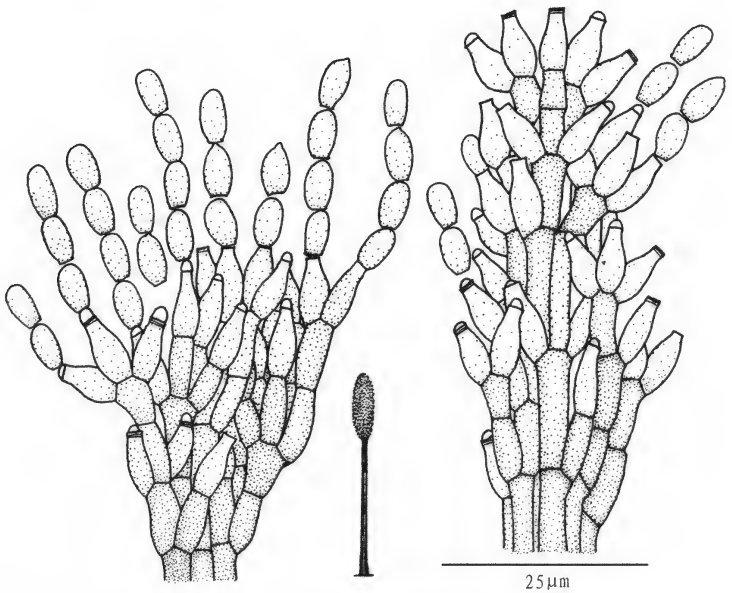

Fig. 7. *Cephalotrichum oblongum*: conidia, conidiogenous cells and synnemata.

Cephalotrichum oblongum J.J. Xu & T.Y. Zhang, **sp. nov.** Fig. 7

MycoBank MB 561113

Coloniae effusae, pulveraceae vel velutinae, griseae, brunneo-virens vel fuscae, reverse fuscae, in PDA crescentes mediocris et attingentes diametrum 6.5 centimetri ad 25°C post duas hebdomadas. Mycelium plerumque superficiale, ex hyphis dilute brunneis vel brunneis, ramosis, septatis, laevibus, 2–2.5 µm crassis, in funiculos floccosos interdum assurgens. Synnemata 90–200 µm alta, capitulo ellipsoidea, 10–21 µm lata. Conidiophora dilute brunnea vel atro-brunnea, laevia, septata, 2–3 µm lata. Celluae conidiogenae

percurrentes (annellophora), ampuliformes, penicilliformiter dispositae, 6–10 × 2–3 µm. Conidia catenata, breve cylindrica vel oblonga, basi truncata, apicem rotundata, brunnea, laevia, 3.5–7.5 × 2–2.5 µm.

Holotype: China. Yunnan Province, from a soil of Pingbian County, 11 October 2004, J.J. Xu (HSAUPII$_{04}$2723, dried culture, **holotype**; HMAS196230, **isotype**).

Etymology: in reference to the oblong conidia.

Colonies on PDA effuse, powdery or velvety, grey, greenish brown to blackish brown with the formation of abundantly sporulating structures and synnemata, reverse blackish brown, growing moderately fast, reaching 6.5 cm diameter at 25°C after two weeks. Mycelium mostly superficial, composed of pale brown to brown, branched, septate, smooth, 2–2.5 µm wide hyphae which may occasionally aggregate forming ropes. Synnemata 90–200 µm high, with ellipsoidal heads, 10–21 µm wide. Conidiophores pale to dark brown, smooth, septate, 2–3 µm wide. Conidiogenous cells (annellophores) ampuliform, penicillately arranged, 6–10 × 2–3 µm. Conidia catenate, short cylindrical or oblong, truncate at the base and rounded at the apex, brown, smooth, 3.5–7.5 × 2–2.5 µm.

Comments: Both *C. purpureofuscum* and *C. microsporum* also produce oblong conidia, which, however, differ in size. In *C. oblongum* annellophores are narrower than those of *C. purpureofuscum* and synnemata are shorter than those of *C. purpureofuscum* and *C. microsporum* (Morton & Smith 1963).

Cephalotrichum ovoideum Y.L. Jiang & T.Y. Zhang, **sp. nov.** Fig. 8

MycoBank MB 561115

Coloniae effusae, floccosae, fuscae, reverse flavo-brunneae. Mycelium partem superficiale et partem immersum, ex hyphis hyalinis, ramosis, septatis, laevibus, 1–2.5 µm crassis. Synnemata 250–800 µm alta, ramaso, capitulo sphaerica vel subsphaerica, stipitata, atro-brunnea, 9–25 µm lata. Celluae conidiogenae percurrentes (annellophora), ampuliformes vel obclavatae, penicilliformiter dispositae, 6–13 µm longae, basi tumidulae 2.5–3.7 µm diam, quae in zonam annellatam, 1.5–2 µm diam. Conidia solitaria vel catenata, dilute flavo-brunnea vel flavo-brunnea, ovoidea, obpyriformia vel subsphaerica, apicem acuta vel interdum rotundata, basin truncata, laevia, 4.5–7 × 3–4 µm.

Holotype: China. Sichuan Province, Jiuzhaigou, from a forest soil, 18 August 2005, Y.L. Jiang (HSAUPII$_{05}$0846, dried culture, **holotype**; HMAS196231, **isotype**).

Etymology: in reference to the ovoid conidia.

Colonies on PDA effuse, at first powdery, finally floccose, blackish brown, reverse yellowish brown. Mycelium partly superficial, partly immersed, composed of hyaline, branched, septate, smooth, 1–2.5 µm wide hyphae. Synnemata 250–800 µm high, branched with spherical or subspherical heads, stipes dark brown, 9–25 µm wide. Conidiogenous cells (annellophores) ampuliform to obclavate, penicillately arranged, 6–13 µm long, swollen base 2.5–3.7 µm diam, tapering abruptly into the annellated zone 1.5–2 µm diam. Conidia solitary

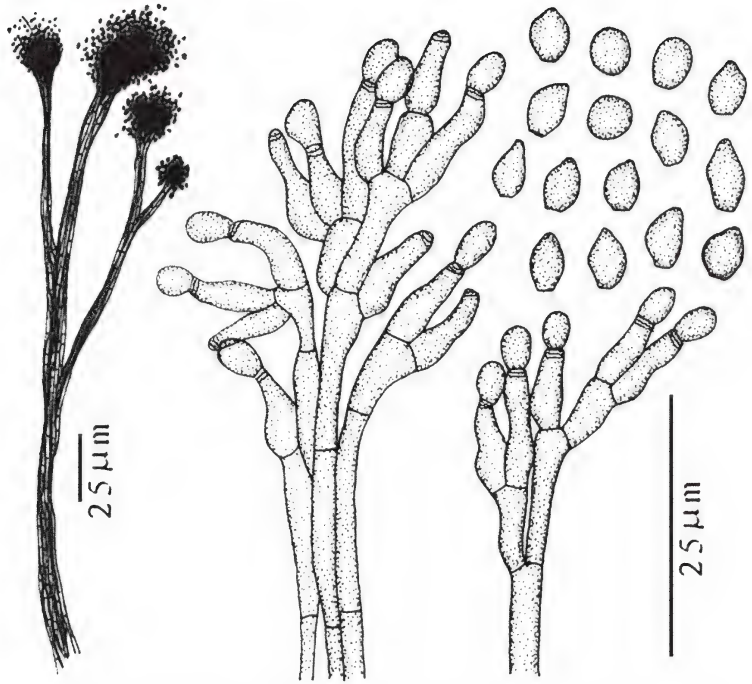

FIG. 8. *Cephalotrichum ovoideum*: conidia, conidiogenous cells and synnemata.

or catenate, pale yellowish brown to yellowish brown, ovoid, obpyriform or subspherical, usually pointed at the apex or sometimes rounded, truncate at the base, smooth, 4.5–7 × 3–4 µm.

COMMENTS: *Cephalotrichum ovoideum* resembles *C. purpureofuscum* and *C. byssoides* in conidial morphology, but the conidia of *C. purpureofuscum* are usually rounded at the apex and its synnemata are unbranched. Conidial ornamentation and size clearly distinguish *C. ovoideum* and *C. byssoides* (Morton & Smith 1963, Mason & Ellis 1953).

Cephalotrichum robustum Y.L. Jiang & T.Y. Zhang, **sp. nov.** FIG. 9

MYCOBANK MB 561116

Coloniae effusae, floccosae, brunneo-virens, reverse flavo-brunnea. Mycelium partem superficiale et partem immersum, ex hyphis hyalinis, ramosis, septatis, laevibus, 1–3 µm crassis. Synnemata 250–750 µm alta, capitulo cylindrica et elongato-ellipsoidea, stipitata, atro-brunnea, 6–25 µm lata. Celluae conidiogenae percurrentes (annellophora), subcylindricae, robustae, penicilliformiter dispositae, 4–8 µm longae, basi tumidulae 2–4 µm diam, zonam annellatam 1.5–2.5 µm diam. Conidia solitaria vel catenata, late ellipsoidea vel subsphaerica, basin truncata, apicem rotundata, flavo-brunnea, laevia, 5–7 × 3–5 µm.

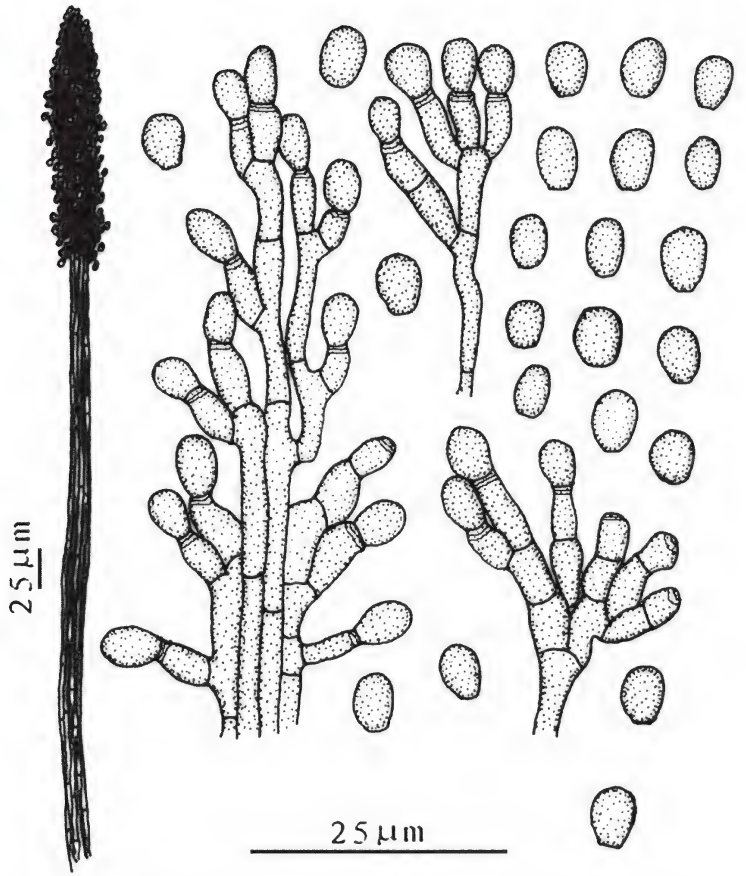

FIG. 9. *Cephalotrichum robustum*: conidia, conidiogenous cells and synnema.

HOLOTYPE: China. Sichuan Province, from a forest soil of Jiuzhaigou, 18 August 2005, Y.L. Jiang (HSAUPII$_{05}$0875, dried culture, **holotype**; HMAS196232, **isotype**).

ETYMOLOGY: in reference to the thickset annellophores.

Colonies on PDA effuse, at first powdery, finally floccose, greenish brown, reverse yellowish brown. Mycelium partly superficial, partly immersed, composed of hyaline, branched, septate, smooth, 1–3 µm wide hyphae. Synnemata 250–750 µm high, with cylindrical to long ellipsoidal heads, stipes dark brown, 6–25 µm wide. Conidiogenous cells (annellophores) subcylindrical, thickset, penicillately arranged, 4–8 µm long, swollen base 2–4 µm diam, the annellated zone 1.5–2.5 µm diam. Conidia solitary or catenate, broadly ellipsoidal to subspherical, truncate at the base and rounded at the apex, yellowish brown, smooth, 5–7 × 3–5 µm.

Comments: *Cephalotrichum robustum* morphologically resembles *C. ellipsoideum*, *C. longicollum*, *C. castaneum* (Jiang & Zhang 2008), and *C. purpureofuscum*. The annellophores of *C. robustum* are the shortest among these five species. In addition, synnemata of *C. robustum* are shorter than in *C. purpureofuscum* and *C. castaneum*, longer than in *C. ellipsoideum* (Ellis 1971), and about the same length as in *C. longicollum*. However, the conidia of *C. robustum* are bigger than those of *C. longicollum*.

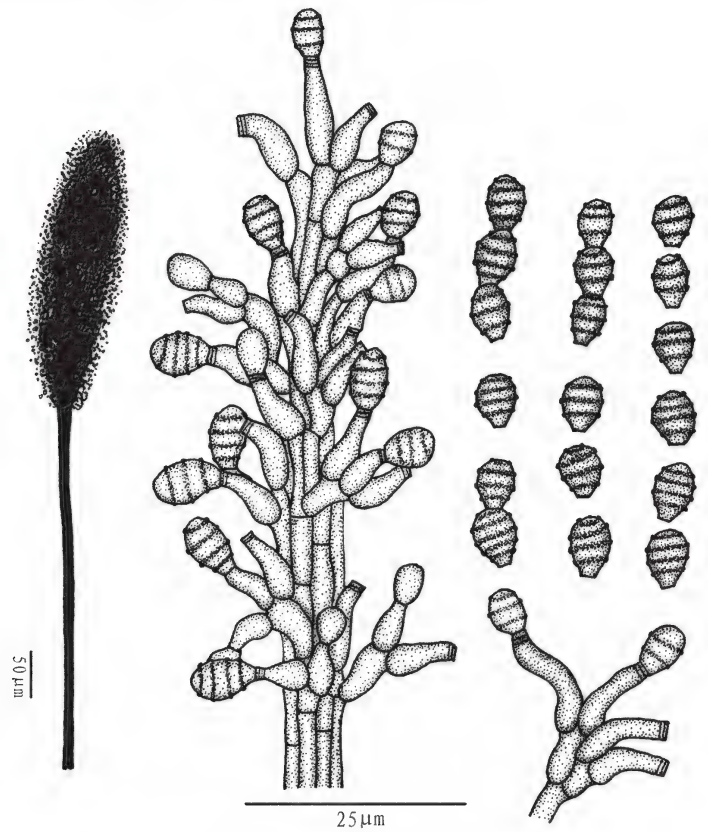

Fig. 10. *Cephalotrichum spirale*: conidia, conidiogenous cells and synnema.

Cephalotrichum spirale H.M. Liu, H.Q. Pan & T.Y. Zhang, **sp. nov.** Fig. 10

MycoBank MB 561117

Coloniae effusae, floccosae, atrogriseae vel atrae, in PDA crescentes lentissime et attingentes diametrum 4 centimetri ad 25°C post duas hebdomadas. Mycelium plerumque superficiale, ex hyphis hyalinis, ramosis, septatis, laevibus, 1–2.5 µm crassis. Synnemata 700 µm alta, capitulo ellipsoidea et cylindrica, stipitata, atro-brunnea, 10–15 µm lata. Celluae

conidiogenae percurrentes (annellophora), ampuliformes vel obclavatae, penicilliformiter dispositae, 6–12 µm longae, basi tumidulae 2–3.5 µm diam, quae in zonam annellatam, 1–2 µm diam. Conidia solitaria vel catenata, brunnea, late obovoidea vel late ellipsoidea, basin truncata et apicem rotundata, spiraliter disposita verruculosa, 5–7 × 3–4.5 µm, in universum fusca.

HOLOTYPE: China. Qinghai Province, from a grassland soil of Dari County, 12 June 2007, H.Q. Pan (HSAUPII$_{07}$4033, dried culture, **holotype**; HMAS196233, **isotype**).

ETYMOLOGY: in reference to the verruculose conidia with verrucae in a spiral.

Colonies on PDA effuse, floccose, central sporulating area at first pale grey, the grey colour spreading and darkening to blackish, finally dark grey to black with the formation of abundantly sporulating structures and synnemata, growing slow, reaching 4 cm diameter at 25°C after two weeks. Mycelium mostly superficial, composed of hyaline, branched, septate, smooth, 1–2.5 µm wide hyphae. Synnemata 700 µm high, with ellipsoidal to cylindrical heads, stipes dark brown, 10–15 µm wide. Conidiogenous cells (annellophores) ampuliform to obclavate, penicillately arranged, 6–12 µm long, swollen base 2–3.5 µm diam, tapering abruptly into the annellated zone 1–2 µm diam. Conidia solitary or catenate, brown, broadly obovoid to broadly ellipsoidal, truncate at the base and rounded at the apex, verruculose, verrucae arranged in a spiral, 5–7 × 3–4.5 µm, blackish brown in mass.

COMMENTS: Based on the presence of ornamented conidia, *C. spirale* is similar to *C. stemonitis*, *C. byssoides*, *C. phillipsii*, *C. nanum* (Ehrenb.) S. Hughes (Hughes 1958), and *C. verrucisporum* (Jiang & Zhang 2008). However, conidial shape distinguishes *C. spirale* from the other five species. In addition, synnemata of *C. spirale* are longer than in *C. phillipsii* and shorter than in *C. stemonitis*, *C. nanum*, *C. byssoides*, and *C. verrucisporum* (Mason & Ellis 1953, Morton & Smith 1963, Jiang & Zhang 2008).

Cephalotrichum terricola Y.L. Jiang & T.Y. Zhang, **sp. nov.** FIG. 11

MYCOBANK MB 561118

Coloniae effusae, floccosae, brunneo-virens, reverse fuscae. Mycelium partem superficiale et partem immersum, ex hyphis hyalinis, ramosis, septatis, laevibus, 1–3 µm crassis. Synnemata 300–660 µm alta, capitulo fusiformia vel elongato-cylindrica, stipitata, atro-brunnea, 9–19 µm lata. Celluae conidiogenae percurrentes (annellophora), ampuliformae, penicilliformiter dispositae, 4.5–9.5 µm longae, basi tumidulae 2–4.5 µm diam, quae in zonam annellatam, 1–2 µm diam. Conidia solitaria vel catenata, obovoidea vel ellipsoidea, flavo-brunnea, laevia, basin truncata, apicem rotundata vel interdum leviter acuta, 4.5–7×2.5–3.5 µm.

HOLOTYPE: China. Sichuan Province, Panzhihua, from a mountain soil, China. 13 August 2005, Y.L. Jiang (HSAUPII$_{05}$0924, dried culture, **holotype**; HMAS196227, **isotype**).

ETYMOLOGY: in reference to the habitat.

Colonies on PDA effuse, at first powdery, finally floccose, greenish brown, reverse blackish brown. Mycelium partly superficial, partly immersed,

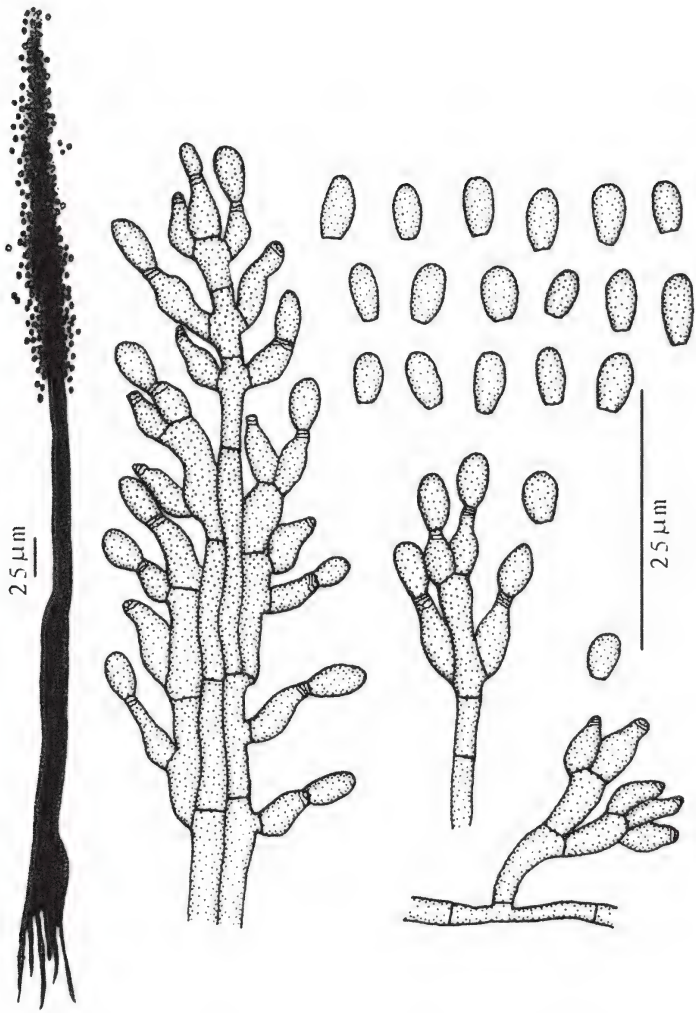

Fig. 11. *Cephalotrichum terricola*: conidia, conidiogenous cells and synnema.

composed of hyaline, branched, septate, smooth, 1–3 µm wide hyphae. Synnemata 300–660 µm high, with fusiform to long cylindrical heads, stipes dark brown, 9–19 µm wide. Conidiogenous cells (annellophores) ampuliform, penicillately arranged, 4.5–9.5 µm long, swollen base 2–4.5 µm diam, tapering abruptly into the annellated zone 1–2 µm diam. Conidia solitary or catenate, obovoid to ellipsoidal, yellowish brown, smooth, truncate at the base, rounded or sometimes slightly pointed at the apex, 4.5–7 × 2.5–3.5 µm.

COMMENTS: The species most similar to *C. terricola* in conidial morphology are *C. ellipsoideum* and *C. purpureofuscum,* except that those species produce wider conidia. Additionally, synnemata of *C. purpureofuscum* are longer (≤ 900 μm) with spherical or subspherical heads and those of *C. ellipsoideum* shorter (100–500 μm) with ellipsoidal to ovoid heads.

Cephalotrichum verrucipes Y.L. Jiang & T.Y. Zhang, **sp. nov.** FIG. 12

MYCOBANK MB 561119

Coloniae effusae, floccosae, brunneo-virens, reverse fuscae. Mycelium partem superficiale et partem immersum, ex hyphis hyalinis, ramosis, septatis, laevibus, 2–4.5 μm crassis. Synnemata 2800 μm alta, capitulo cylindrica vel ellipsoidea, stipitata, dilute brunnea, verruculosa, 25–66 μm lata. Conidiophora verruculosa. Celluae conidiogenae percurrentes (annellophora), longa ampuliformae vel obclavata, penicilliformiter disposita, 7–14 μm longa, basi leviter tumidula 1–2.5 μm diam, quae in zonam annellatam, 1–2 μm diam. Conidia solitaria vel catenata, sphaerica, subsphaerica vel ellipsoidea, laevia, dilute flavo-brunnea vel flavo-brunnea, 2–3.5 × 2–3 μm, in universum atro-brunnea.

HOLOTYPE: China. Sichuan Province, from a forest soil of Jiuzhaigou, 19 August 2005, Y.L. Jiang (HSAUPII$_{05}$0849, dried culture, **holotype**; HMAS196234, **isotype**).

ETYMOLOGY: in reference to the verruculose conidiophores.

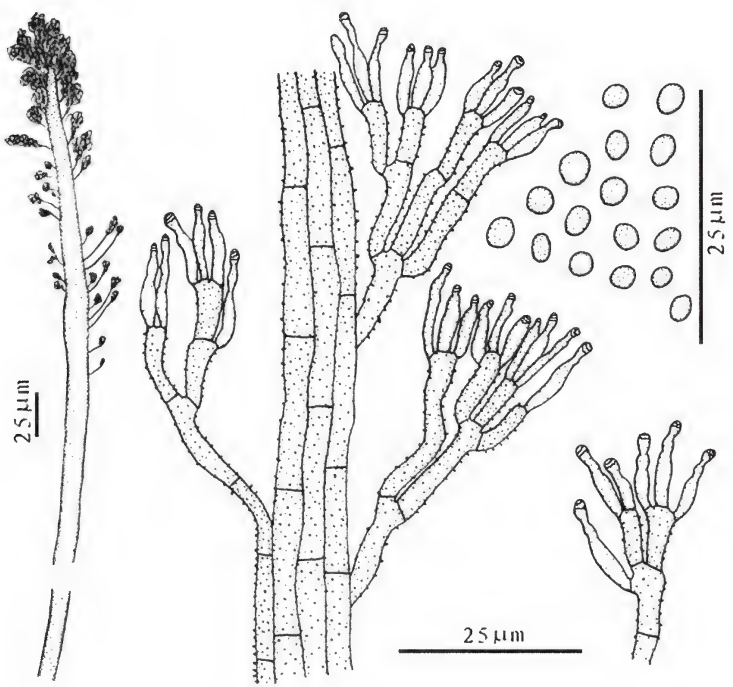

FIG. 12. *Cephalotrichum verrucipes*: conidia, conidiogenous cells and synnema.

Colonies on PDA effuse, at first powdery, finally floccose, greenish brown, reverse blackish brown. Mycelium partly superficial, partly immersed, composed of hyaline, branched, septate, smooth, 2–4.5 µm wide hyphae. Synnemata 2800 µm high, with cylindrical vel ellipsoidal heads, stipes pale brown, verruculose, 25–66 µm wide, stipes distinct, but density of branches of stipes nonuniform, disperse branches often in the middle part or below the stipes. Conidiophores verruculose. Conidiogenous cells (annellophores) long ampuliform to obclavate, penicillately arranged, 7–14 µm long, slightly swollen base 1–2.5 µm diam, tapering abruptly into the annellated zone 1–2 µm diam. Conidia solitary or catenate, spherical, subspherical to ellipsoidal, smooth, pale yellowish brown to yellowish brown, 2–3.5 × 2–3 µm, dark brown in mass.

Comments: *Cephalotrichum verrucipes* is the only species in the genus with verruculose conidiophores.

New combinations

The following two species, previously placed in *Doratomyces,* both produce spores in a dry head at the apex of a complex, erect condiophore synnema. For that reason we transfer them to *Cephalotrichum.*

Cephalotrichum castaneum (Y.L. Jiang & T.Y. Zhang) Y.L. Jiang & T.Y. Zhang, **comb. nov.**

MycoBank MB 561107

≡ *Doratomyces castaneus* Y.L. Jiang & T.Y. Zhang, Mycotaxon 104: 131, 2008.

Cephalotrichum verrucisporum (Y.L. Jiang & T.Y. Zhang) Y.L. Jiang & T.Y. Zhang, **comb. nov.**

MycoBank MB 561120

≡ *Doratomyces verrucisporus* Y.L. Jiang & T.Y. Zhang, Mycotaxon 104: 133, 2008.

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Morphology and phylogeny of *Pseudocercospora kamalii* sp. nov., a foliar pathogen on *Terminalia* from India

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Abstract — *Pseudocercospora kamalii* (*Mycosphaerellaceae, Capnodiales*) is associated with severe leaf spot disease of *Terminalia chebula* (*Combretaceae*) in natural forests of Mahabaleshwar in the Western Ghats of India. Morphological characterization reveals that the new species proposed has some affinity with the species of genus *Prathigada*. However, based on the molecular sequence data (LSU and ITS 1-5.8S-ITS 2) generated in the present study, we established its identity as a species of *Pseudocercospora*. The species is also compared morphologically with other *Pseudocercospora* species reported on *Terminalia* from different parts of the world.

Key words — anamorphic fungi, *Mycosphaerella*, plant pathogen

Introduction

The microfungal diversity of northern Western Ghats has been well explored in the recent past (Karandikar & Singh 2010, Rajeshkumar et al. 2010, 2011; Singh et al. 2009, 2010; Waingankar et al. 2008). During January 2011 a survey was conducted to explore the microfungal diversity in the natural forests of Mahabaleshwar, situated in the northern part of the Western Ghats, India, at 17°58′N 73°43′E. An unusual synnematous cercosporoid species was discovered that caused a severe foliar disease in *Terminalia chebula*. The present study aimed to identify the causal agent of the foliar infection on *Terminalia* in northern Western Ghats, India using morphological and molecular methods.

Materials & methods

Isolates and morphology— Synnemata of the fungus were directly isolated from the surface of fallen fruits and observed under a Nikon Binocular stereo microscope (Model SMZ-1500 with Digi-CAM, Japan). Single conidial cultures were established

on 2% potato dextrose agar plates (PDA; Crous et al. 2009a). For morphotaxonomic studies and photomicrographs an Olympus (Model CX-41, Japan) microscope was used. Conidia and conidiomata were mounted in lactic acid cotton blue and measured using an ocular micrometer, with 30 observations per structure. Colony characteristics in culture were studied on two different media: 2% malt extract agar (MEA) and PDA (Crous et al. 2009a). Herbarium specimens were deposited in the Ajrekar Mycological Herbarium (AMH); the culture was accessioned and preserved in the National Fungal Culture Collection of India (NFCCI; WDCM-932), Agharkar Research Institute, Pune, India under accession number NFCCI 2344.

Polymerase chain reaction (pcr) and sequencing— Total DNA was extracted from cultures grown on PDA plates for two weeks at 25 °C, using a FastDNA® SPIN kit as per the manufacturer's instructions (MP Biomedicals GmbH, Germany). Fragments containing the region encoding the 28S nrDNA (LSU) and ITS 1-5.8S nrDNA-ITS 2 (ITS) were amplified using primer pairs LROR (Rehner & Samuels 1994) and LR7 (Vilgalys & Hester 1990) for LSU; ITS4 and ITS5 (White et al. 1990) for ITS. DNA amplification was performed in a 25 µl reaction using 2 µl of template DNA (10–25 ng), 0.5 U of Taq DNA polymerase (Genei, Bangalore, India), 2.5 µl of 10× Taq DNA polymerase buffer, 0.5 µl of 200 µM of each dNTPs (Genei, Bangalore, India), 0.5 µl of 10 pmol primer, H_2O (Sterile Ultra Pure Water, Sigma) to make up 25 µl. Amplification in an Eppendorf Mastercycler AG used the following parameters: 5 min at 95 °C; 30 cycles of 1 min at 95 °C, 30s at 56 °C, and 1 min at 72 °C for the ITS region amplification; and final 7-min extension step at 72 °C. DNA amplification of LSU followed the ITS conditions except for a 52 °C annealing temperature. The PCR products were purified with an Axygen PCR cleanup kit (Axygen Scientific Inc, CA, USA) and sequenced with the same primers using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). The sequencing reactions were run on an ABI 3100 automated DNA sequencer (Applied Biosystems, USA).

Sequence alignment and phylogenetic analysis— LSU & ITS sequences from *P. kamalii* were aligned manually using the text editor option of the Molecular Evolutionary Genetics Analysis (MEGA) software v4.0. (Tamura et al. 2007). The manually edited NFCCI 2344 sequences were deposited in the NCBI sequence nucleotide database (ITS: JF 824126, LSU: JF 824127). They were also subjected to a BLAST search of the NCBI Genbank nucleotide database. The ITS sequences were aligned using Clustal W together with the homologous regions of ITS of closely related species of *Pseudocercospora* Speg. and *Mycosphaerella* s.l. For ITS, the matrix was analyzed with the Maximum Parsimony

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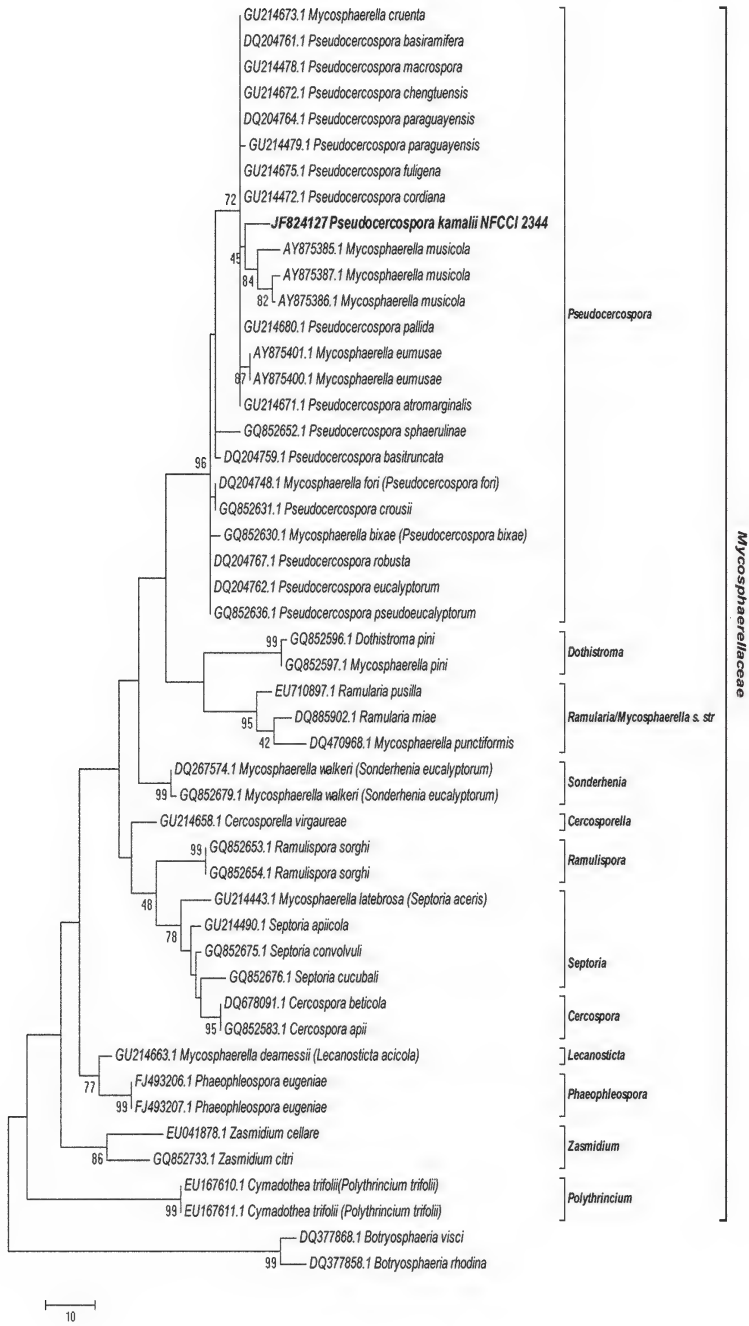
Fig. 1. Phylogenetic tree based on aligned LSU sequences of *Pseudocercospora* and its teleomorph, *Mycosphaerella* s.l. The consistency index (0.566343), the retention index (0.821571), and the composite index (0.479675) were calculated in MEGA for all sites and parsimony-informative sites (in parentheses). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level 3 in which the initial trees were obtained with the random addition of sequences (10 replicates). The tree is drawn to scale, with branch lengths calculated using the average pathway method and are in the units of the number of changes over the whole sequence. All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option).


GU214673.1 Mycosphaerella cruenta
DQ204761.1 Pseudocercospora basiramifera
GU214478.1 Pseudocercospora macrospora
GU214672.1 Pseudocercospora chengtuensis
DQ204764.1 Pseudocercospora paraguayensis
GU214479.1 Pseudocercospora paraguayensis
GU214675.1 Pseudocercospora fuligena
GU214472.1 Pseudocercospora cordiana
JF824127 Pseudocercospora kamalii NFCCI 2344
AY875385.1 Mycosphaerella musicola
AY875387.1 Mycosphaerella musicola
AY875386.1 Mycosphaerella musicola
GU214680.1 Pseudocercospora pallida
AY875401.1 Mycosphaerella eumusae
AY875400.1 Mycosphaerella eumusae
GU214671.1 Pseudocercospora atromarginalis
GQ852652.1 Pseudocercospora sphaerulinae
DQ204759.1 Pseudocercospora basitruncata
DQ204748.1 Mycosphaerella fori (Pseudocercospora fori)
GQ852631.1 Pseudocercospora crousii
GQ852630.1 Mycosphaerella bixae (Pseudocercospora bixae)
DQ204767.1 Pseudocercospora robusta
DQ204762.1 Pseudocercospora eucalyptorum
GQ852636.1 Pseudocercospora pseudoeucalyptorum
GQ852596.1 Dothistroma pini
GQ852597.1 Mycosphaerella pini
EU710897.1 Ramularia pusilla
DQ885902.1 Ramularia miae
DQ470968.1 Mycosphaerella punctiformis
DQ267574.1 Mycosphaerella walkeri (Sonderhenia eucalyptorum)
GQ852679.1 Mycosphaerella walkeri (Sonderhenia eucalyptorum)
GU214658.1 Cercosporella virgaureae
GQ852653.1 Ramulispora sorghi
GQ852654.1 Ramulispora sorghi
GU214443.1 Mycosphaerella latebrosa (Septoria aceris)
GU214490.1 Septoria apiicola
GQ852675.1 Septoria convolvuli
GQ852676.1 Septoria cucubali
DQ678091.1 Cercospora beticola
GQ852583.1 Cercospora apii
GU214663.1 Mycosphaerella dearnessii (Lecanosticta acicola)
FJ493206.1 Phaeophleospora eugeniae
FJ493207.1 Phaeophleospora eugeniae
EU041878.1 Zasmidium cellare
GQ852733.1 Zasmidium citri
EU167610.1 Cymadothea trifolii(Polythrincium trifolii)
EU167611.1 Cymadothea trifolii (Polythrincium trifolii)
DQ377868.1 Botryosphaeria visci
DQ377858.1 Botryosphaeria rhodina
Pseudocercospora
Dothistroma
Ramularia/Mycosphaerella s. str
Sonderhenia
Cercosporella
Ramulispora
Septoria
Cercospora
Lecanosticta
Phaeophleospora
Zasmidium
Polythrincium
Mycosphaerellaceae
10

method using the Tamura model (Tamura et al. 2007) to calculate the sequence divergence, and the bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option in MEGA4).

Results

DNA phylogeny

The alignment for the *Pseudocercospora* LSU phylogenetic analyses comprised a total of 481 positions in the final dataset, of which 128 were parsimony informative. The LSU sequence analysis presented here (Fig. 1) reveals the systematic position of the new species within *Pseudocercospora,* along with its 11 closely allied sister genera in *Mycosphaerellaceae.* The LSU phylogenetic sequence analysis implied 12 main clades: *Pseudocercospora*, *Dothistroma* Hulbary, *Ramularia* Unger/*Mycosphaerella* s. str., *Sonderhenia* H.J. Swart & J. Walker, *Cercosporella* Sacc., *Ramulispora* Miura, *Septoria* Sacc., *Cercospora* Fresen., *Lecanosticta* Syd., *Phaeophleospora* Rangel, *Zasmidium* Fr., and *Polythrincium* Kunze. All *Pseudocercospora* species clustered together to form a major clade. The new species, *P. kamalii,* clustered close to *Mycosphaerella musicola* R. Leach ex J.L. Mulder in the major *Pseudocercospora* clade. *Botryosphaeria visci* (Kalchbr.) Arx & E. Müll. (DQ 377868.1) and *B. rhodina* (Berk. & M.A. Curtis) Arx (DQ 377858.1) in the *Botryosphaeriaceae* served as outgroup taxa.

For the phylogenetic analysis of closely related *Pseudocercospora* species, the ITS sequence data alignment of 487 bp included 452 positions in the final data set. The analysis of ITS sequence shown in Fig. 2 reveals a significant association of *Pseudocercospora* representatives, some with *Mycosphaerella*-like teleomorphs. The ITS sequence analysis implied four major clades, with *Pseudocercospora eumusae* Crous & Mour. (= *Mycosphaerella eumusae* Crous & Mour.) forming a unique clade (94% bootstrap support). However, *P. tereticornis* Crous & Carnegie, *P. cruenta* (Sacc.) Deighton, *P. pallida* (Ellis & Everh.) H.D. Shin & U. Braun, *P. casuarinae* Crous & R.G. Shivas, *P. elaeodendri* (G.P. Agarwal & Hasija) Deighton, *P. fuligena* (Roldan) Deighton, *P. chengtuensis* (F.L. Tai) Deighton, and *P. atromarginalis* (G.F. Atk.) Deighton clustered together to form a major group in the *Mycosphaerellaceae*. *Pseudocercospora musae* (Zimm.) Deighton (= *Mycosphaerella musicola*) also formed a unique clade (100% bootstrap support). Our new species, *P. kamalii,* (66% bootstrap support), separated as sister to the *Pseudocercospora musae* (AY 646475.1 & AY 646474.1) clade. *Cladosporium herbarum* (Pers.) Link (= *Davidiella tassiana* (De Not.) Crous & U. Braun) (DQ 289799.2) was chosen as the outgroup, as it belongs to the family *Davidiellaceae* that is allied to the *Mycosphaerellaceae* (Crous et al. 2006).

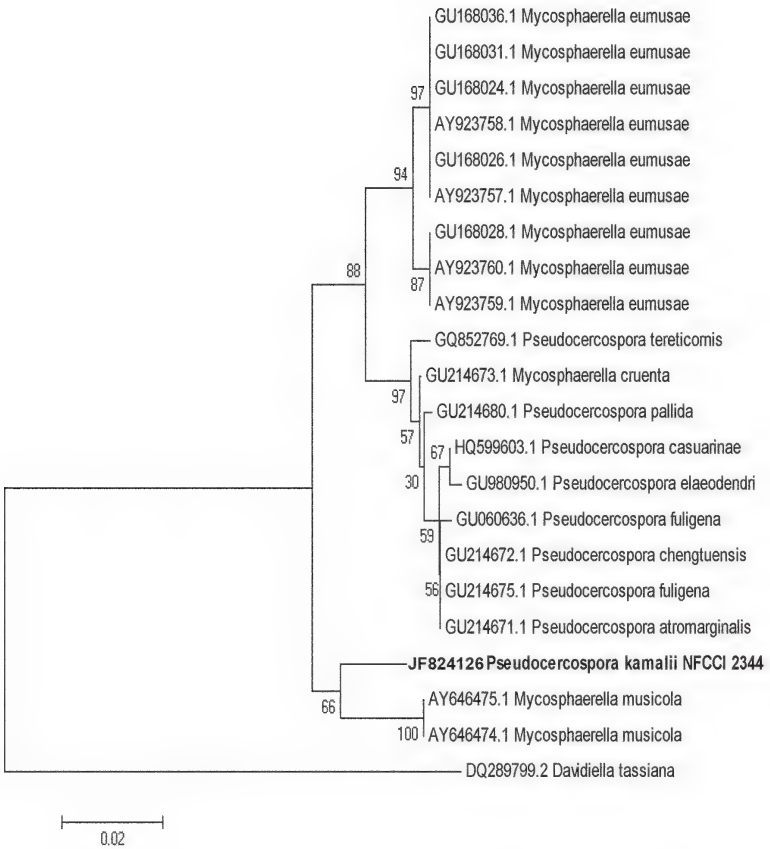


FIG. 2. Phylogenetic tree based on aligned ITS sequences of *Pseudocercospora* and its teleomorph, *Mycosphaerella* s.l. and inferred using the Neighbor-Joining method in MEGA. The optimal tree with the sum of branch length = 0.25106088 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete Deletion option).

Taxonomy

The new cercosporoid species on *Terminalia chebula* is easily distinguishable from all *Pseudocercospora* species hitherto described on *Terminalia* by its synnematous conidiomata, and hence it is described here as new species.

Pseudocercospora kamalii Rajeshkumar, Rahul Sharma & S.K. Singh, **sp. nov.**

MycoBank MB 561201 Figs. 3–4

Synnemata 200–337.5 µm longa, ex stromatibus oriunda. Stromata bene evoluta, globosa, subglobosa vel irregularia, atrobrunnea. 50–72 µm diam., ex cellulis oblongis, cylindraceis composita. Conidiophora 130–263 × 5–6 µm, modice brunnea vel atrobrunnea, crassitunicata, pluriseptata. Conidia solitaria, sicca, in multitudine atro-brunnea, 25–57.5 × 5.5–8 µm, laevia, raro verruculosa, crassitunicata, 2–7 septis crassis, basi truncate.

Type: India, Mahabaleshwar, Western Ghats, Maharashtra, on leaves of *Terminalia chebula* Retz. (*Combretaceae*), January 2011, K.C. Rajeshkumar & Rahul Sharma (AMH 9425, **holotype**; ex-type culture NFCCI 2344.)

Etymology: *kamalii*, named in honour of Prof. Dr. Kamal, Emeritus Professor, Department of Botany, D.D.U. Gorkhpur University for his major contribution to the cercosporoid fungi of India.

Leaf spots necrotic, amphigenous, circular, angular or irregular, forming concentric dark and pale brown patterns on the spots, spreading eventually covering most of the leaf, but not vein limited, 1–3 cm diam. Conidiomata synnematous and caespituli either exclusively hypogenous or amphigenous, synnemata blackish brown 200–337.5 µm long, arising from well developed stroma, globose, subglobose or irregular, dark brown or blackish brown, 50–72 µm diam, formed of elongated cylindrical cells. Conidiophores medium to dark brown, thick and dark-walled, formed as a continuation of stromatic cells, 130–263 µm long and 5–6 µm wide, 8–10 to multiseptate. Conidiogenous cells integrated, terminal, cylindrical or sometime doliiform, geniculate, slightly wider and paler towards truncate apex, which at times can be obtusely rounded; conidial scars 1–2 in number, 2–3 µm diam, slightly thicker and dark, conidial loci not usually protuberant. Conidia solitary, holoblastic, dry, dark brown to blackish brown in mass, pale brown when immature, later turning to medium to dark brown, 25–57.5 × 5.5–8 µm, straight or slightly curved, mostly smooth, rarely minutely verruculose, thick-walled, thick septate, 2–7-septate, conidial wall and septations are thicker and darker in the basal part, and pale and thin towards apex, conidial base truncate, 2.0–2.2 µm, and apex obtuse or subacute 2.5–3.5 µm

Teleomorph: not observed.

Colonies on MEA (Fig. 5) very slow growing, 0.5 to 0.8 mm diam after 7 days, grayish white or white initially, later turning dark grayish black to blackish brown, velutinous, reverse blackish to dark blackish brown. Colonies become 2 to 2.3 mm after 30 days, forming a sulcate pattern, and sectored on MEA.

Fig. 3. *Pseudocercospora kamalii* (holotype). a. Habit. b. Symptoms on leaves and synnemata. c–d. Synnemata side view. e. Synnemata top view. f–h. Conidioma with stroma. i. Well developed stroma. j. Conidiophore tip and conidial scar. k–m. Conidia with thick wall and thick septations. Bars: k, l, m = 25 µm.

b
c
d
e
f
g
h
i
j
k
l
m
25µm

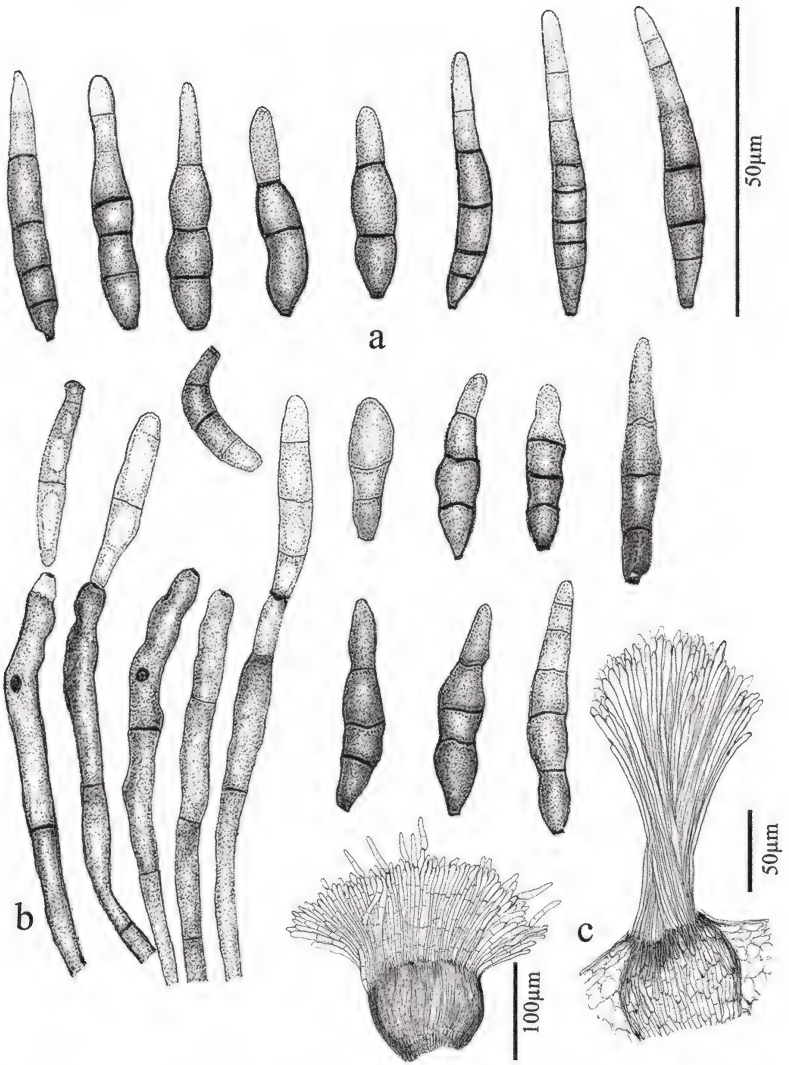


FIG. 4. *Pseudocercospora kamalii*. (holotype). a. Conidiophore and conidial development. b. Conidia. c. Synnemata

Culture on MEA not sporulating (lacking conidial development) but forming distinct synnemata-like bunches of conidiophores and dark celled stroma-like structures. The hyphae are medium to dark brown, highly verruculose and interwoven, forming a thick mat.

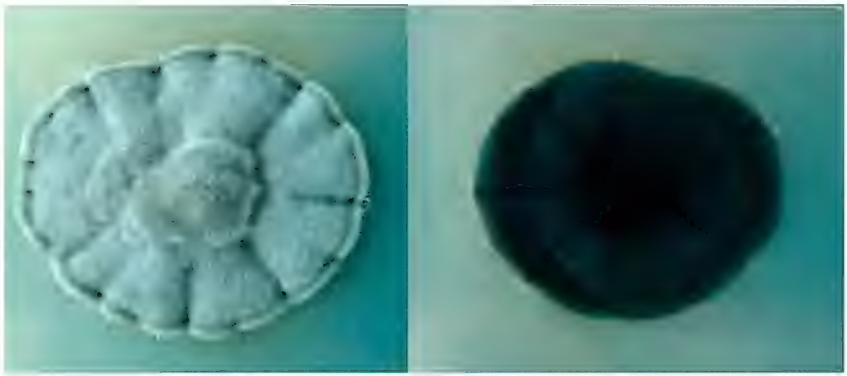


FIG. 5. *Pseudocercospora kamalii.* (holotype) Ex-type culture on MEA. a. Top view b. Reverse view.

Discussion

The new species, *Pseudocercospora kamalii,* is morphologically quite unusual and obscure within *Pseudocercospora,* as it exhibits many characters that match the genus *Prathigada.* Among these, the thick conidial septations and wall pattern are mostly identical with species of *Prathigada*. Only a few cercosporoid species with similar morphological characters (thick-walled, multi-septate, pigmented conidia containing slightly thickened and darkened scars) have been transferred to *Prathigada* (Braun 1996; Furlanetto & Dianese 1999; Sutton 1994), most being retained in *Pseudocercospora.* Thus far, 15 species are recorded under the genus *Prathigada.* Initial morphological studies also made it evident that *Pseudocercospora kamalii* is morphologically close to *Prathigada terminaliae* (Syd.) B. Sutton recorded on *Terminalia* spp. (Sutton 1994). However, *P. kamalii* has synnematous conidiomata, smaller conidia, and fewer conidial septa. Furthermore, the LSU and ITS sequence analyses accurately defined the species boundaries and placed *P. kamalii* into *Pseudocercospora.* Crous et al. (2009b) recently stated that due to the unavailability of cultures, no decision could yet be made on the phylogenetic placement of several less well-known genera such as *Prathigada* Subram. within the *Mycosphaerellaceae.* A recollection, epitypification and detailed phylogenetic analysis of the type species, *Prathigada cratevae* (Syd.) Subram. on *Crateva religiosa* G. Forst. from India, is needed to reveal the exact relation of the genus *Prathigada* with *Pseudocercospora* and their placement in *Mycosphaerellaceae.*

Sutton (1994) also reported six *Pseudocercospora* taxa on *Terminalia* spp. from India: *P. arjunae* B. Sutton (on *T. arjuna*), *P. brevis* B. Sutton (on *T. bellerica*), *P. catappae* (Henn.) Y.L. Guo & X.J. Liu (on *T. catappa*, *T. tomentosa*, *T. arjuna*), *P. chebulae* B. Sutton (on *T. chebula*), *P. combretacearum*

R.K. Verma & Kamal var. *combretacearum* (on *T. bellerica*, *Terminalia* sp.), and *P. combretacearum* var. *minima* B. Sutton (on *T. bellerica*); he also reported two other species: *P. neodeightonii* B. Sutton (on *T. albida* from Sierra Leone) and *P. zambiensis* (Deighton) B. Sutton (on *T. mollis* from Zambia). Kamal (2010: 347) listed *P. catappae, P. chebulae,* and *P. combretacearum* var. *combetacearum* from Indian *T. chebula.* These three species differ from *P. kamalii* in various morphological characters; most conspicuously, they have mononematous, fasciculate, or sporodochial conidiophores in contrast with the synnematous conidiophores of *P. kamalii*, which is the only synnematous *Pseudocercospora* species recorded on *Terminalia*.

In the present study, *P. kamalii* is proposed as a new species based on the LSU & ITS sequence analysis (Fig. 1, 2) and morphological characterization (Fig. 3, 4). Phylogenetically, *P. kamalii* is closely related to *Pseudocercospora musae* (66% bootstrap support) on *Musa* spp. (Arzanlou et al. 2008, Braun et al. 1999). With its distinct synnemata with a well-developed basal stroma and larger conidiophores and conidia, *P. kamalii* is morphologically distinct from *Pseudocercospora musae* and all other taxa with higher phylogenetic similarity.

Acknowledgements

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Mycena sect. *Longisetae*: a new species, a new name, and an addition

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Abstract — *Mycena saloma* sp. nov. is described and illustrated from Kerala State, India. *Mycena lomavritha* nom. nov. is proposed for *Mycena indica* Manim. & Leelav., nom. illegit. The Brazilian species *M. rhaphidocephala,* previously accepted in sect. *Sacchariferae*, is accepted in sect. *Longisetae.*

Keywords — *Agaricales, Basidiomycota,* nomenclature, taxonomy

Introduction

As currently accepted, *Mycena* sect. *Longisetae* A.H. Sm. ex Maas Geest. (*Basidiomycota, Agaricales, Mycenaceae*) incorporates about eleven species that are primarily characterised by several long, needle-like, thick-walled hairs on the pileus (Desjardin et al. 2002). Additionally, these species have ascending, narrowly adnate to free lamellae, a pruinose to hispid stipe with a typically discoid base, ellipsoid basidiospores, 4-spored basidia, a hymenium devoid of pleurocystidia, a pileus margin often beset with cystidia, dextrinoid pileal and stipe trama, smooth caulocystidia that typically taper towards the apex, and a pileipellis composed of either acanthocysts or spinulose hyphae. According to Desjardin et al. (2002), southeast Asia could be the centre of diversity of this group.

During our investigations on the agaric mycota of Kerala State, India, we came across an as yet undescribed species belonging to this group that we describe here. *Mycena indica*, a species originally placed by Manimohan & Leelavathy (1988) in sect. *Sacchariferae* and subsequently transferred to the amended sect. *Longisetae* by Desjardin et al. (2002), needs a replacement name (nom. nov.) as the present one is an illegitimate later homonym (McNeill et al. 2006: Art. 53.1). A nom. nov. for this species is proposed here, along with some observations on the species. The Brazilian species *M. rhaphidocephala,*

originally placed by Maas Geesteranus & de Meijer (1998) in sect. *Sacchariferae*, has all the characteristics of sect. *Longisetae* and is accepted in the latter section here.

Materials & methods

Conventional morphology-based taxonomic methods were employed for this study. Microscopic observations were made on material stained with 1% aqueous solution of Congo red and mounted in 3% aqueous KOH. Melzer's reagent was used to observe whether the spores and tissues were amyloid. For evaluation of the range of spore-size, twenty basidiospores each from one specimen of each collection cited were measured. The herbarium acronyms follow Thiers (2011). The collections cited without herbarium acronyms are in the personal herbarium of the second author. The concept of Desjardin et al. (2002) is followed for *Mycena* sect. *Longisetae*.

Taxonomy

Mycena saloma Aravind. & Manim., **sp. nov.** PLATE 1

MYCOBANK MB 519881

Basidiomata delicata, dispersa. Pileus 1.25–5 mm latus, hemisphericus vel convexus, transluso-striatus, subsulcatus, albus, pilis erectis subtilis vestitus. Lamellae adnexae, albae, subdistantae, lamellularum intermixtae. Stipes 3.5–22 × 0.25–0.5 mm, centralis, cylindricus, transluso-albus, puberulus, basi discoideo instructus. Sporae 7.5–9.5(–11) × 3.75–5.5 µm, inaequilateraliter ellipsoideae, amyloideae, leves. Basidia 12–19 × 9.5–13 µm, subglobosa et pedicellata, 4-sporigera. Cheilocystidia et pleurocystidia nulla. Trama pilei et lamellarum ex hyphis dextrinoidei composita. Hyphae pileipellidis 2–11.5 µm latae, echinulatae, spinulis minutis instructae. Pileosetae 60–780 × 15–30 µm (ad basim) × 10–11 µm (ad apicem), hyalinae, crassitunicatae. Cystidia margine pilei 17–82 × 6.5–20 µm, clavata vel fusoidea, tenuitunicata, spinulosa. Caulocystidia 92.5–125.5 × 15–33 µm, flexuoso-cylindrica vel fusoidia, hyalina, tenuitunicata, leves. Hyphae omnes fibulatae.

TYPE: INDIA, Kerala State, Idukki District, Munnar, Kanniamalai Estate: 14.VIII.2010, **holotype:** Dollymol DM461 (L).

ETYMOLOGY: *saloma* (Sanskrit), hairy

Basidiomata very small, delicate. Pileus 1.25–5 mm diam., up to 3 mm high, initially parabolic, becoming hemispherical to convex with age; surface initially pure white, becoming off-white with age, with prominent white hairs up to 0.5 mm long that are denser at the centre, pellucid–striate, very faintly sulcate, dry, finely pruinose; margin straight, finely fringed. Lamellae adnexed, pure white, thin, 0.5–0.75 mm wide, subdistant, with lamellulae of 1–2 lengths; edge entire, concolourous with the sides. Stipe 3.5–22 × 0.25–0.5 mm, central, terete, tapering towards apex, hollow; surface translucent white, pubescent, more so towards the base, almost glabrous at apex; base discoid, with finely radiating basal mycelium. Context very thin, concolourous with the pileus surface. Odour and taste not distinctive.

Plate 1: *Mycena saloma*. A, basidioma (holotype); B, basidiospores (DM486); C, basidium (DM486); D, pileus marginal cystidium (DM486); E, caulocystidium (DM486); F, pileipellis (DM486); G, pileus hair (DM486). (Scale bars: A = 5 mm; B–G = 10 µm.)

Basidiospores 7.5–9.5(–11) × 3.75–5.5 (8.57 ± 0.68 × 4.45 ± 0.44) µm, Q = 1.66–2.4, Qm = 1.9, pip-shaped to ovo-ellipsoid, thin-walled, hyaline, smooth, with refractive guttules, strongly amyloid. Basidia 12–19 × 9.5–13 µm, subglobose, pedicellate, hyaline, bearing 4 sterigmata up to 5 µm long. Lamella-edge fertile. Cheilocystidia and pleurocystidia not seen. Lamellar trama regular to subregular; hyphae 2–15.5 µm, often inflated up to 30 µm, thin-walled, hyaline, faintly dextrinoid in Melzer's reagent; subhymenium pseudoparenchymatous. Pileus trama subregular; hyphae 3–26 µm wide, rarely inflated up to 37.5 µm, thin-walled, hyaline, distinctly dextrinoid in Melzer's reagent. Pileipellis a cutis of repent hyphae supporting scattered pileal hairs; hyphae 2–11.5 µm wide, thin- to slightly thick-walled (0.25 µm), hyaline, spinulose, with acanthophysoid terminal elements; excrescences 0.5–1.5 × 0.25–0.5 µm; acanthophysoid terminal elements 12–37 × 7–19 µm, repent or ascending, clavate to narrowly clavate or cylindrical, with refractive guttules and with excrescences 0.5–2 × 0.5–1 µm; pileal hairs 60–780 µm long, 15–30 µm broad at the base, 10–11 µm broad at the apex, gradually tapering towards the obtuse apex, smooth, hyaline, with a refractive wall 1–9 µm thick. Pileus margin beset with marginal cystidia; marginal cystidia 17–82 × 6.5–20 µm, clavate or fusiform, entirely spinulose or often with a smooth apical prolongation, thin-walled, hyaline; excrescences 0.75–4 × 0.75–1 µm. Stipitipellis a cutis; hyphae 2–8 µm wide, thin-walled, hyaline, smooth. Caulocystidia 92–125 × 15–33 µm, flexuoso-cylindric, fusoid or lageniform with a subconical apex, thin-walled, hyaline, smooth. Stipe trama strongly dextrinoid in Melzer's reagent. Clamp connections present on all hyphae.

Habitat: On dicotyledonous leaves and twigs, scattered; altitude: 1800–2000 m.

Additional specimen examined: **INDIA, Kerala State**, Idukki District, Munnar, Eravikulam National Park: 16.VIII.2010, Dollymol DM486 (L).

Discussion: This species has all the diagnostic features of sect. *Longisetae* discussed earlier. The key by Desjardin et al. (2002) to species of *Mycena* sect. *Longisetae* leads to a couplet differentiating *M. breviseta* Höhn. and *M. longiseta* Höhn. *Mycena breviseta* is a poorly known species whose holotype collection is reported to contain 'no intact basidiomes and no pilei' (Desjardin et al. 2002). The Desjardin et al. (2002) key describes this Javan species as having an umbilicate pileus, pileal hairs that are up to 1000 µm long, and clavate marginal cystidia that are entirely spinulose. The closely related *M. longiseta,* also from Java, has a grey pileus and pileal hairs that are up to 1000 µm long. A perusal of *Mycena* literature revealed that the Brazilian species, *M. rhaphidocephala* (whose infrageneric position is discussed below but hitherto placed in sect. *Sacchariferae*), is strikingly similar to *M. saloma* in most macro- and microscopic features. An examination of the *M. rhaphidocephala* holotype

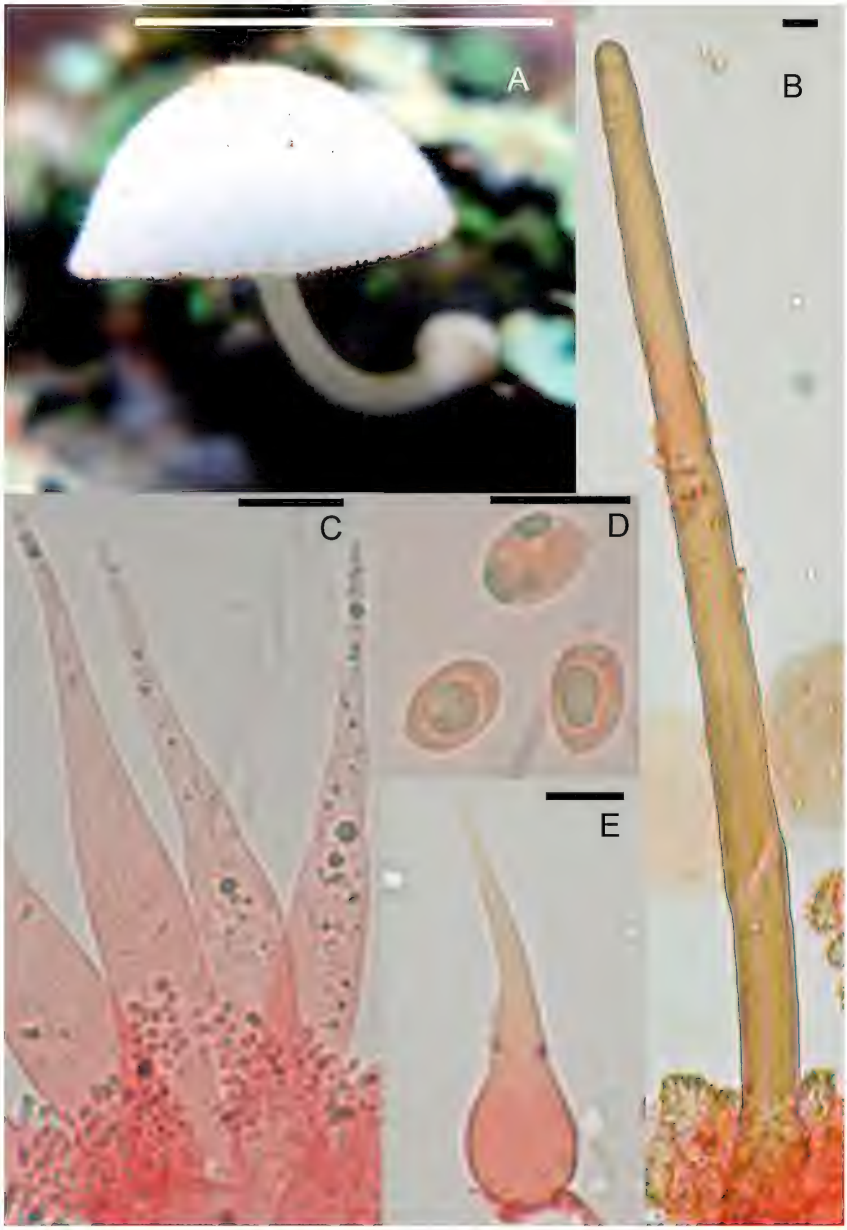

Plate 2: *Mycena lomavritha*. A, basidioma (AF70); B, pileus hair (DM423); C, cheilocystidia (DM423); D, basidiospores (DM423); E, caulocystidium (DM423).

(Scale bars: A = 5 mm; B–E = 10 µm.)

and protologue, however, revealed that it has pileal hairs that are much longer (up to 3000 µm), smooth pileipellis hyphae, well-developed acanthocysts, consistently lageniform caulocystidia, and clampless hyphae.

Mycena lomavritha Manim., **nom. nov.** PLATE 2

MYCOBANK MB 519882

≡ *Mycena indica* Manim. & Leelav., Mycologia 80(6): 861 (1989, "1988"), nom. illeg., non Sarwal & Rawla 1983.

ETYMOLOGY: *lomavritha* (Sanskrit), covered with hair.

DESCRIPTION AND ILLUSTRATIONS: Manimohan & Leelavathy (1989).

SPECIMENS EXAMINED: **INDIA**, **KERALA STATE**, MALAPPURAM DISTRICT, Calicut University campus: 6.VII.1987, Manimohan M383 (holotype TRTC 50991); 7.VI.2006, Dollymol DM13; PALAKKAD DISTRICT, Silent Valley National Park: 16.VI.2010, Dollymol DM423.

DISCUSSION: Thus far known only from southern India, *M. lomavritha* was originally described based on a single collection. Several collections were subsequently made by the present authors from Kerala State, India. All our observations support the original description except that the pileal trama was consistently dextrinoid. Also, while the original collection was found growing on the bark of a jackfruit tree, we found the species also growing on the bark of other trees. *Mycena lomavritha* is characterised by small off-white corticolous basidiomata with a hairy pileus and discoid stipe base, amyloid basidiospores, dextrinoid tramal tissues, ventricose-fusoid cheilocystidia with sparsely spinulose lower middle region, a pileipellis composed of acanthocysts as well as scattered thick-walled, aculeate hairs, and abundant obclavate caulocystidia.

Manimohan & Leelavathy (1988) placed this species in sect. *Sacchariferae* despite the thick-walled pileus hairs because sect. *Longisetae* then was characterized by inamyloid basidiospores, gelatinized pileipellis, and absence of clamp connections. Subsequently both sect. *Sacchariferae* (Desjardin 1995) and sect. *Longisetae* (Desjardin & Horak 2002) were redefined. As discussed by Desjardin et al. (2002), the combination of characters exhibited by *M. lomavritha* are clearly better accommodated in the redefined sect. *Longisetae,* where it seems close to *M. brunneisetosa* Corner from Singapore.

Mycena rhaphidocephala Maas Geest. & de Meijer, Persoonia 17: 39 (1998). PLATE 3

DESCRIPTION AND ILLUSTRATION: Maas Geesteranus & de Meijer (1998: 39–40, Fig. 7).

SPECIMEN EXAMINED: **BRAZIL**, **PARANA STATE**, Morretes, Porto do Cima, Parque Murumbi: 27.VI.1995, A.A.R. de Meijer MA-3098 (L, holotype).

DISCUSSION: This Brazilian species has all the characteristic features of the redefined sect. *Longisetae* such as the small off-white basidiomata, thick-walled aculeate hairs on the pileus, discoid stipe base, ellipsoid basidiospores,

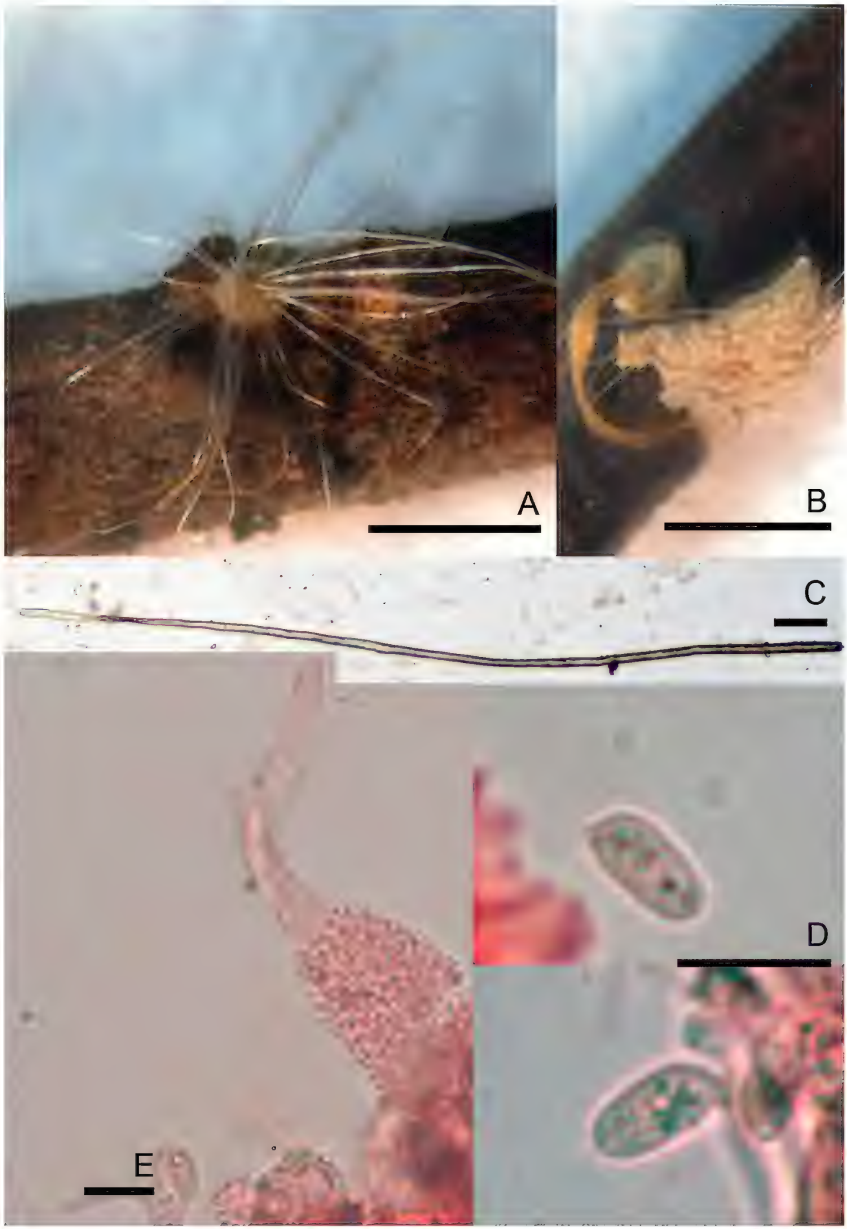

Plate 3: *Mycena rhaphidocephala* (holotype). A, primordium; B, mature basidioma; C, pileus hair; D, basidiospores; E, pileus marginal cystidia.

(Scale bars: A, B = 1 mm; C = 100 µm; D, E = 10 µm.)

acanthocysts on the pileipellis, spinulose marginal cystidia with a smooth apical prolongation, and smooth caulocystidia that taper towards the apex. We have verified these features on the holotype of this species. What Maas Geesteranus & de Meijer (1998) had described as cherocytes are actually the spinulose marginal cystidia typical of the sect. *Longisetae* (see Desjardin 1995: 5 for the history and definition of the term cherocyte). Our examination of these entities in the holotype revealed that they are thin-walled —not "somewhat thick-walled" as noted by Maas Geesteranus & de Meijer (1998)— and hence cannot be called cherocytes. Maas Geesteranus & de Meijer (1998), who originally placed this species in sect. *Sacchariferae,* noted that its combination of characters was not known in any other species of that section. We accept *M. rhaphidocephala* as a member of sect. *Longisetae.*

Acknowledgment

We are indebted to Prof. D.E. Desjardin and Dr D.J. Lodge for presubmission reviews of our manuscript. We are also thankful to Dr M.E. Noordeloos and the staff of the Nationaal Herbarium, Leiden Branch, for arranging a loan of the holotype of *Mycena rhaphidocephala*. Mr V. Adnaan Farook is thanked for letting us use his field photograph of *M. lomavritha* in this paper.

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Three new hyphomycetes from southern China

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Abstract – Three new hyphomycetes were collected from dead branches from tropical and subtropical forests in southern China: *Ellisembia schimae* from *Schima superba*, *Linkosia hibisci* from *Hibiscus mutabilis*, and *Stanjehughesia micheliae* from *Michelia skinneriana*. The new species are described, illustrated, and compared with similar species.

Key words – anamorphic fungi, taxonomy

Introduction

The mycota of tropical and subtropical forests of southern China is very rich in wood-inhabiting fungi (Ma et al. 2008, Dai et al. 2009, Dai & Li 2010, Zhang et al. 2009a). During ongoing mycological surveys in these forests, three hyphomycetes with morphological features typical of *Ellisembia* Subram., *Stanjehughesia* Subram. (Subramanian 1992), and *Linkosia* A. Hern. Gut. & B. Sutton (Hernández-Gutiérrez & Sutton 1997) were collected on dead branches. However, these collections differ significantly from the currently accepted species of these genera, and are therefore proposed as new taxa. The specimens are deposited in the Herbarium of Shandong Agricultural University, Plant Pathology (HSAUP) and the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS).

Taxonomy

Ellisembia schimae Jian Ma & X.G. Zhang, **sp. nov.** Fig. 1

MycoBank MB 561476

Fungus anamorphicus. Coloniae in substrato naturali effusae, brunneae vel atrobrunneae, pilosae. Mycelium partim superficiale, partim immersum in substrato, ex hyphis ramosis, septatis, pallide brunneis, laevibus, 1.5–3 µm crassis compositum. Conidiophora macronemata, mononematica, singula vel fasciculata, erecta, nonramosa, recta vel flexuosa, cylindrica, atrobrunnea vel nigra, laevia, septata, 16–39 × 6.5–9 µm. Cellulae conidiogenae monoblasticae, integratae, terminales, determinatae, lageniformes

Fig. 1. *Ellisembia schimae.*
A. Conidiophores with conidia. B. Conidiophores. C–D. Conidia.

vel cylindricae, brunneae vel atrobrunneae, laeves. Conidiorum secessio schizolytica. Conidia *holoblastica, solitaria, acrogena, recta vel curvata, obclavata, laevia, brunnea vel atrobrunnea, 14–21-distoseptata, 122–155 μm longa, 4.5–6.5 μm crassa, apicem acutum gradatim versus attenuata, basi truncata 3.5–4.5 μm lata.*

Type: China, Guangdong Province: Liuxihe National Forest Park, on dead branches of *Schima superba* Gardn. & Champ. (*Theaceae*), 16 Oct 2010, J. Ma, (**holotype** HSAUP H5380–2; isotype HMAS146141).

Etymology: in reference to the host genus, *Schima.*

Anamorphic fungi. Colonies on natural substrate effuse, brown to dark brown, hairy. Mycelium partly superficial, partly immersed, composed of branched, septate, pale brown, smooth-walled hyphae, 1.5–3 μm thick. Conidiophores differentiated, single or in groups, erect, unbranched, straight or flexuous, cylindrical, dark brown to black, smooth, septate, 16–39 × 6.5–9 μm. Conidiogenous cells monoblastic, integrated, terminal, determinate,

lageniform or cylindrical, brown to dark brown, smooth. Conidial secession schizolytic. Conidia holoblastic, solitary, acrogenous, straight or curved, obclavate, smooth-walled, brown to dark brown, 14–21-distoseptate, 122–155 µm long, 4.5–6.5 µm thick in the broadest part, tapered gradually toward the acute apex, 3.5–4.5 µm wide at the truncate base.

Comments – Subramanian established the genus *Ellisembia,* proposing twelve new combinations for twelve species of *Sporidesmium* Link and designating *E. coronata* (Fuckel) Subram. as the type species (Subramanian 1992). Subsequently, Wu & Zhuang (2005) merged *Imicles* Shoemaker & Hambl. (Shoemaker & Hambleton 2001) into *Ellisembia* and expanded the generic concept. *Ellisembia* was mainly characterized by differentiated, single, unbranched, septate conidiophores, and monoblastic, integrated, terminal, lageniform, doliiform or cylindrical, determinate or percurrently extending conidiogenous cells that produce solitary, holoblastic, distoseptate conidia. Assigning species within *Ellisembia* is primarily based on conidial features such as shape, size and septation (Subramanian 1992, Wu & Zhuang 2005, McKenzie 2010).

Our specimen is clearly a species of *Ellisembia* from its conidiophore and conidial morphology. The new species bears some resemblance to *E. britannica* (B. Sutton) W.P. Wu and *E. carrii* (Morgan-Jones) W.P. Wu (Wu & Zhuang 2005) in conidial shape. However, *E. schimae* differs from *E. britannica* (conidia 50–130 × 3–5 µm, 10–13-distoseptate) in its larger conidia with more numerous septa, and from *E. carrii* (conidia 90–130 × 8–10 µm) in having longer and narrower conidia. In addition, conidia of *E. schimae* have an obvious pointed apex, while those of *E. britannica* and *E. carrii* have rounded apices.

Linkosia hibisci Jian Ma & X.G. Zhang, **sp. nov.** Fig. 2

MycoBank MB 561477

Fungus anamorphicus. Coloniae in substrato naturali effusae, brunneae. Mycelium superficiale, ex hyphis ramosis, septatis, brunneis vel atrobrunneis, laevibus, 1.5–3 µm crassis compositum. Conidiophora nulla. Cellula conidiogena determinatae, monoblastica, solitaria, simplicia, lageniformia vel ampulliformia, brunnea vel atrobrunnea, laevia, 9–22 × 4–5 µm, ad apicem 4–4.5 µm crassa et truncatae. Conidiorum secessio schizolytica. Conidia holoblastica, solitaria, acrogena, recta vel curvata, obclavata vel obclavata-rostrata, atrobrunnea vel brunnea, laevia, 16–21-distoseptata, 160–210 µm longa, 7.5–9.5 µm crassa, apicem versus ad 1.5–2.5 µm attenuata; cellula apicalis pallide brunnea, rotundata; cellula basalis cylindrica vel conico-truncata, ad basim 2–4.5 µm crassa.

Type: China, Hainan Province: tropical forest of Bawangling, on dead branches of *Hibiscus mutabilis* L. (*Malvaceae*), 8 Dec 2010, J. Ma (**holotype** HSAUP H5199–1; isotype HMAS 146142).

Etymology: in reference to the host genus, *Hibiscus.*

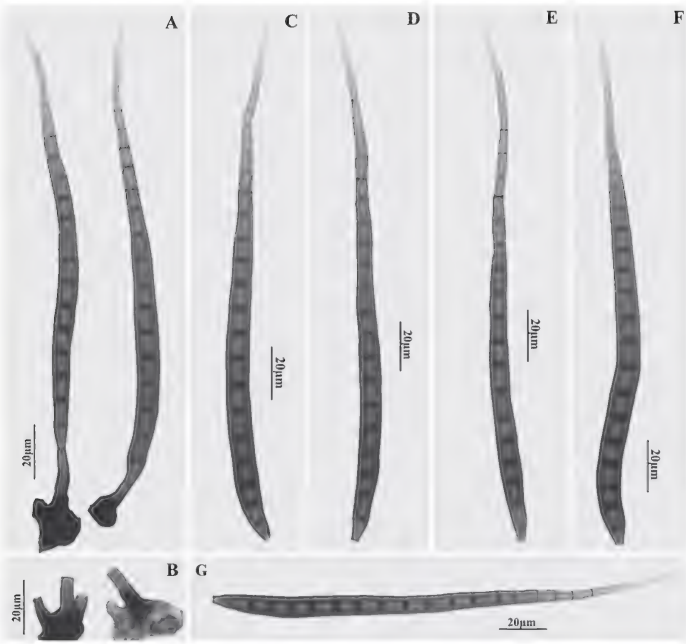

Fig. 2. *Linkosia hibisci.*
A. Conidiogenous cells with conidia. B. Conidiogenous cells. C–G. Conidia.

Anamorphic fungi. Colonies on natural substrate effuse, brown. Mycelium superficial, composed of branched, septate, brown to dark brown, smooth-walled hyphae, 1.5–3 μm thick. Conidiophores absent. Conidiogenous cells determinate, monoblastic, solitary, simple, lageniform or ampulliform, brown to dark brown, smooth, 9–22 × 4–5 μm, 4–4.5 μm wide at the truncate apex. Conidial secession schizolytic. Conidia holoblastic, solitary, acrogenous, straight or curved, obclavate to obclavate-rostrate, dark brown to brown, smooth, 16–21-distoseptate, 160–210 μm long, 7.5–9.5 μm thick in the broadest part, tapering to 1.5–2.5 μm near the apex; apical cells pale brown, rounded; basal cell cylindrical, truncate, 2–4.5 μm wide.

Comments—The genus *Linkosia*, with *L. coccothrinacis* (A. Hern. Gut. & J. Mena) A. Hern. Gut. & B. Sutton as the type species, was characterized by absence of conidiophores, and solitary, simple, short, monoblastic, determinate, lageniform or ampulliform conidiogenous cells producing distoseptate conidia (Hernández-Gutiérrez & Sutton 1997, Wu & Zhuang 2005). These characters separate *Linkosia* from other similar genera including *Stanjehughesia* and *Janetia* M.B. Ellis (Ellis 1976). Six species are currently included in this genus, of

which four have previously been described from China (Hernández-Gutiérrez & Sutton 1997, Wu & Zhuang 2005, Zhang et al. 2009b). All six species are reported to survive saprobically on dead leaves or branches of bamboo.

Of the known species, *Linkosia hibisci* is similar to *L. obclavata* W.P. Wu (Wu & Zhuang 2005) and *L. mori* K. Zhang & X.G. Zhang (Zhang et al. 2009) in conidial shape, but differs in conidial dimensions (*L. obclavata* 122–177 × 12–16 µm, *L. mori* 110–125 × 9–12 µm). Moreover, *L. hibisci* conidia have more septa than those of *L. obclavata* (12-14 distosepta). In addition, the mature conidia of *L. hibisci* are more darkly pigmented than those of the other two species.

Stanjehughesia micheliae Jian Ma & X.G. Zhang, **sp. nov.** FIG. 3

MycoBank MB 561478

Fungus anamorphicus. Coloniae in substrato naturali effusae, nigrae. Mycelium superficiale, ex hyphis ramosis, septatis, pallide brunneis vel brunneis, laevibus, 1.5–3 µm crassis compositum. Conidiophora nulla vel brevis, 1–3-septata, brunnea vel atrobrunnea, 11–28 × 4.5–5 µm. Cellula conidiogena monoblastica, determinatae, solitaria, simplicia, lageniformia vel ampulliformia, brunnea vel atrobrunnea, laevia, 4.5–6.5 × 3.5–5 µm, ad apicem 3–4.5 µm crassa et truncatae. Conidiorum secessio schizolytica. Conidia holoblastica, solitaria, acrogena, recta vel curvata, obclavata vel obclavata-rostrata, atrobrunnea vel brunnea, laevia, 13–19-distoseptata, 130–190 µm longa, 7–9 µm crassa, apicem versus ad 2–3 µm attenuata; cellula apicalis rotundata; cellula basalis cylindrica vel conico-truncata, ad basim 3.5–4.5 µm crassa; Appendicibus lateralibus 0–2, brunneae, septata, cylindricae, surgentibus ex cellulla e apicem 2nd vel 3rd.

Type: China, Guangdong Province: Chebaling National Nature Reserve, on dead branches of *Michelia skinneriana* Dunn (*Magnoliaceae*), 16 Oct 2010, J. Ma (**holotype** HSAUP H5414; isotype HMAS146143).

Etymology: in reference to the host genus, *Michelia*.

Anamorphic fungi. Colonies on natural substrate effuse, black. Mycelium superficial, composed of branched, septate, pale brown to brown, smooth-walled hyphae, 1.5–3 µm thick. Conidiophores absent or short, 1–3-septate, brown to dark brown, 11–28 × 4.5–5 µm. Conidiogenous cells monoblastic, determinate, solitary, simple, lageniform or ampulliform, brown to dark brown, smooth, 4.5–6.5 × 3.5–5 µm, 3–4.5 µm wide at the truncate apex. Conidial secession schizolytic. Conidia holoblastic, solitary, acrogenous, straight or curved, obclavate to obclavate-rostrate, dark brown to brown, smooth, 13–19-distoseptate, 130–190 µm long, 7–9 µm thick in the broadest part, tapering to 2–3 µm near the apex; apical cells rounded; basal cell cylindrical, truncate, 3.5–4.5 µm wide; lateral appendages 0–2, brown, septate, cylindrical, arising from the 2nd or 3rd cells from the apex.

Comments – The genus *Stanjehughesia* was established by Subramanian (1992) to accommodate five new combinations from *Sporidesmium*. The

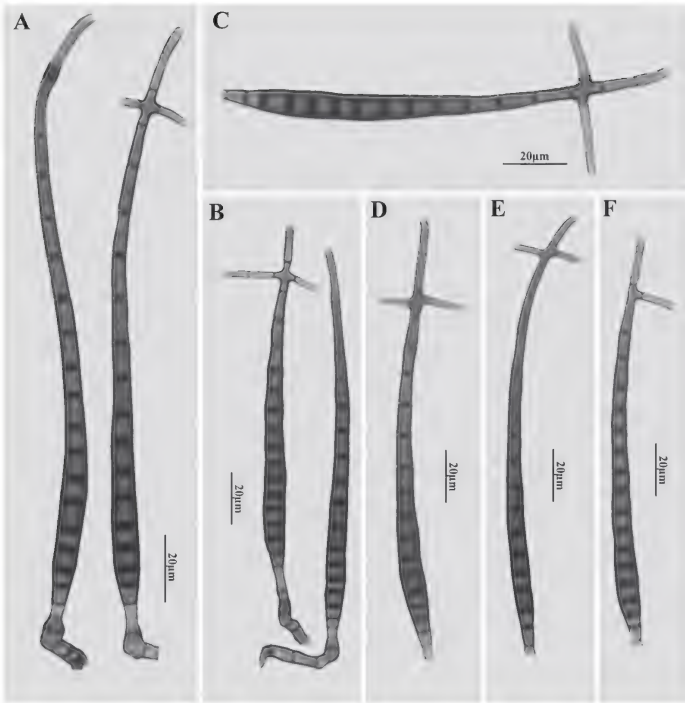

FIG. 3. *Stanjehughesia micheliae*.
A–B. Reduced conidiophores or conidiogenous cells with conidia. C–F. Conidia.

genus is characterized by very reduced or absent conidiophores; determinate, monoblastic, lageniform or ampulliform, solitary, short, simple conidiogenous cells that produce obclavate to obclavate-rostrate, euseptate conidia. Additional species have been described or transferred to *Stanjehughesia* (McKenzie 1995, Mena-Portales et al. 2001, Wu & Zhuang 2005, Delgado 2008, Marincowitz 2008). At present, the genus comprises 11 species, most of which grow as saprobes on rotten wood, dead branches, stems or decaying leaves.

Stanjehughesia micheliae is unique among known species of *Stanjehughesia* by its obclavate to obclavate-rostrate conidia with 0–2 cylindrical appendages arising from the 2nd or 3rd cell from the apex.

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MYCOTAXON

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A new species of *Junghuhnia* (*Basidiomycota, Meruliaceae*) from tropical China

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Abstract — A new polypore, *Junghuhnia minor* sp. nov., is described and illustrated from the tropical forest of Yunnan Province, southwestern China. The new species is characterized by very thin annual resupinate basidiocarps, a cream to pale buff pore surface with small and slightly lacerated pores, small (2.7–3 × 1.9–2.2 µm) ellipsoid basidiospores, and skeletocystidia.

Key words — lignicolous fungi, *Meruliaceae*, taxonomy

Introduction

Lignicolous fungi are important components of forest ecosystems, where they decompose forest litter and maintain energy flow and material circulation (Lonsdale et al. 2008). Many, including some traditionally used for medicinal purposes in Asia, are edible while others are plant pathogens (Dai et al. 2007, 2009b). New species have been described after recent investigations on lignicolous fungi in subtropical and tropical forests in China (Cui & Dai 2008, Cui et al. 2008, 2011, Dai et al. 2009a, 2010, Dai & Li 2010, Du & Cui 2009, Li & Cui 2010, Li et al. 2008, Wei & Dai 2008, Xiong & Dai 2008, Zhou & Dai 2008).

Characters noted for two polypores collected on fallen angiosperm twig during a survey of the lignicolous fungi in southern China (dimitic hyphal system, encrusted skeletocystidia, small ellipsoid basidiospores) suggest an affinity with *Junghuhnia* Corda. They differ morphologically from existing *Junghuhnia* species, and so I describe and illustrate them as a new species.

Materials & methods

The specimens are deposited at the biological herbarium of Institute of Applied Ecology, Chinese Academy of Sciences (IFP). The microscopic procedure follows Dai

(2010). The microscopic studies were made from sections mounted in Cotton Blue (CB): 0.1 mg aniline blue dissolved in 60 g pure lactic acid; CB+ = cyanophily, CB– = acyanophily. Amyloid and dextrinoid reactions were tested in Melzer's reagent (IKI): 1.5 g KI (potassium iodide), 0.5 g I (crystalline iodine), 22 g chloral hydrate, aq. dest. 20 ml; IKI– = neither amyloid nor dextrinoid reaction. 5% KOH was used as reagent. Sections were studied at magnifications up to ×1000 using a Nikon Eclipse E600 microscope and phase contrast illumination, and dimensions were estimated subjectively with an accuracy of 0.1 μm. In the spore measurements, the apiculus was excluded. In presenting the variation of spore size, 5% of the measurements out of each end of the range are given in parentheses. The following abbreviations are used in the text: L = mean spore length (arithmetical mean of all spores), W = mean spore width (arithmetical mean of all spores), Q = extreme values of the length/width ratios among the studied specimens, and n = the number of spores measured from a given number of specimens. Special color terms are from Anonymous (1969) and Petersen (1996).

Taxonomy

Junghuhnia minor H.S. Yuan, **sp. nov.** FIG. 1

MycoBank MB 561532

Carpophorum annuum, resupinatum. Facies pororum cremeum vel bubalinum; pori angulati, 5–6 per mm. Systema hypharum dimiticum, hyphae generatoriae fibulatae, hyphae skeletales subiculi 2–4 μm diam. Sporae hyalinae, ellipsoideae, 2.7–3 × 1.9–2.2 μm.

TYPE. — China. Yunnan Province, Mengla County, Wangtianshu Forest Park, on fallen angiosperm twig, 16.IX.2007 Yuan 3268 (**holotype** in IFP).

ETYMOLOGY — *minor* (Lat.): refers to small basidiospores.

FRUITBODY — Basidiocarps annual, resupinate, coriaceous, without special odor or taste when fresh, corky when dry, up to 15 cm long, 1 cm wide and 1 mm thick; sterile margin thinning out, membranous, cream, up to 3 mm wide. Pore surface cream to pale buff-yellow upon drying; pores angular, 5–6 per mm, dissepiments thin, slightly lacerate. Subiculum very thin to almost lacking, cream to pale buff, ca. 0.1 mm thick. Tubes concolorous with pore surface, corky, ca. 0.4 mm long.

HYPHAL STRUCTURE — Hyphal system dimitic; generative hyphae bearing clamp connections, skeletal hyphae IKI–, CB+; tissue unchanged in KOH.

SUBICULUM — Dominated by skeletal hyphae; generative hyphae hyaline, thin-walled, occasionally branched, 1.8–3.5 μm diam; skeletal hyphae hyaline, thick-walled with a distinct lumen to subsolid, straight to flexuous, occasionally branched, interwoven, 2–4 μm diam.

TUBES — Generative hyphae infrequent, hyaline, thin-walled, occasionally branched, 1.5–2.5 μm diam; skeletal hyphae dominant, hyaline, thick-walled with a distinct lumen to subsolid, occasionally branched, interwoven, 1.8–2.8

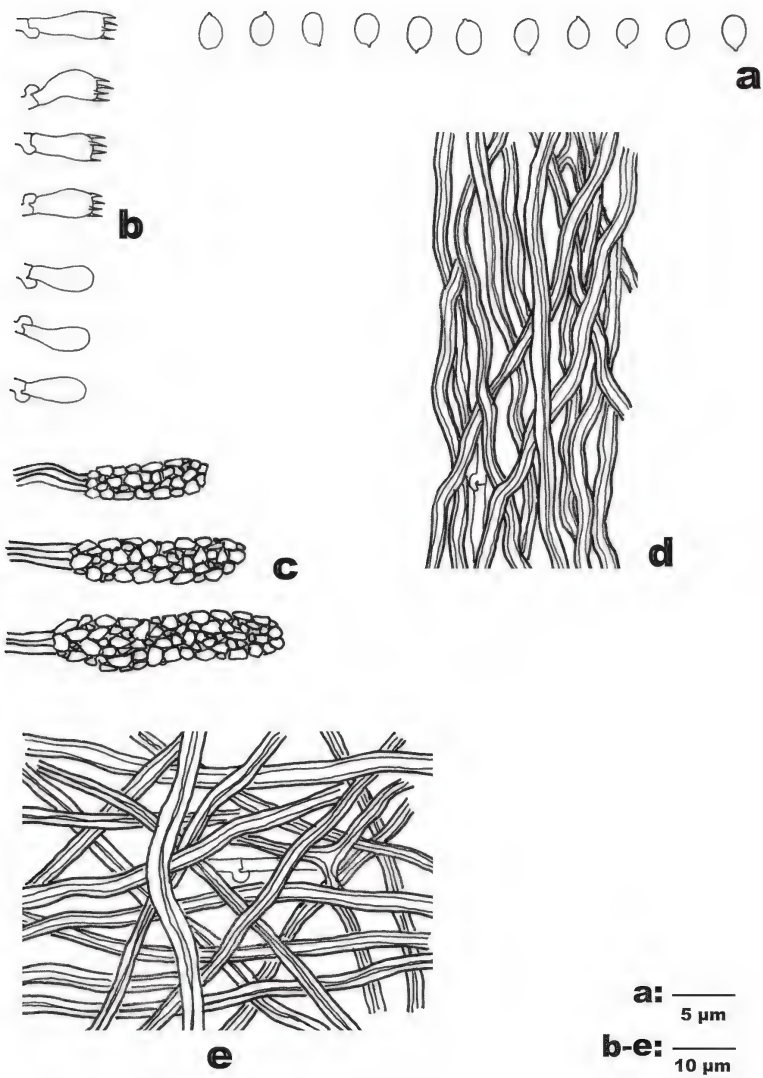

Fig. 1. Microscopic structures of *Junghuhnia minor* (drawn from the holotype).
a: Basidiospores. b: Basidia and basidioles. c: Skeletocystidia.
d: Hyphae from trama. e: Hyphae from subiculum.

μm diam. Skeletocystidia numerous, clavate, thick-walled, originated from trama, embedded or projecting, heavily encrusted, 15–50 × 6–11 μm (with encrustation); basidia clavate, bearing four sterigmata and a clamp connection at the base, 9–12 × 4–5 μm; basidioles in shape similar to basidia, but slightly smaller.

Spores — Basidiospores small, ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, (2.5–)2.7–3 × (1.8–)1.9–2.2(–2.3) μm, L = 2.85 μm, W = 2.04 μm, Q = 1.38–1.41 (n=60/2).

Additional specimen examined — **China. Yunnan Province**, Mengla County, Wangtianshu Forest Park, on fallen angiosperm twig, 16.IX.2007 Yuan 3215 (IFP).

Type of rot — White rot.

Remarks — *Junghuhnia minor* is characterized by annual, resupinate and very thin basidiocarps, pale buff-yellow pore surface with white to cream margin, small ellipsoid basidiospores (2.7–3 × 1.9–2.2 μm), and skeletocystidia.

Junghuhnia luteoalba (P. Karst.) Ryvarden also is characterized by resupinate, pale-buff colored basidiocarps and slightly lacerate dissepiments, but differs in its cylindrical basidiospores and larger pores (4–8 per mm) (Núñez & Ryvarden 2001). In addition, *J. luteoalba* is distributed in boreal and temperate zones.

Junghuhnia crustacea (Jungh.) Ryvarden resembles the new species in its resupinate, thin basidiocarps and cream to pale-buff pore surface but has more shallow pores, distinctly lacerate pores and larger basidiospores (Núñez & Ryvarden 2001, Ryvarden & Johansen 1980).

Junghuhnia minuta I. Lindblad & Ryvarden, which has similar basidiospores (2.5–3 × 2–2.5 μm), differs from *J. minor* by having distinctly pileate basidiocarps and tiny pores (10–12 per mm, Lindblad & Ryvarden 1999).

Junghuhnia microspora Rajchenb. also has small basidiospores but is distinguished from *J. minor* by its orange to chestnut colored pore surface and narrower basidiospores (2.6–3.6 × 1.0–1.6 μm, Rajchenberg 1983).

Other specimens examined: ***Junghuhnia crustacea*** — **China. Yunnan Province**, Mengla County, Wangtianshu Forest Park, on fallen angiosperm branch, 17.IX.2007 Yuan 3638 (IFP); **Kenya. Western Province**, Kakamega district, Kakamega Forest, substrate unknown, 20.I.1970 Ryvarden 5431 (O); **Thailand. Cangwat Chiang Mai**, Amphoe Mae Rim, Kong Hae, on fallen angiosperm trunk, 15.II.1979 Ryvarden 17568 (O).

J. microspora — **Argentina. Missiones**, Iguazu Falls, Apepu Reservation, substrate unknown, 4.III.1982 Rajchenberg 3504 (O).

J. minuta — **Puerto Rico. Luquillo**, Beasley water shed, on deciduous tree, 6.VI.1997 Ryvarden 40279 (O).

J. luteoalba — **China. Heilongjiang Province**, Ning'an County, Volcano forest, on fallen trunk of *Populus*, 14.IX.2004 Yuan 618 (IFP); **Liaoning Province**, Kuandian County, Baishilazi Nat. Res., on rotten wood of *Quercus*, 1.VIII.2004 Cui 1090 (IFP).

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Caloplaca allochroa (lichenized *Ascomycetes*), a new saxicolous lichen species from South Korea

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Abstract — The saxicolous lichen species, *Caloplaca allochroa*, is described as new from subtropical inland localities of Gangwon and Jeollanam Province (South Korea). It is characterized by occurring as two colour variants, yellowish and gray, based on presence vs. absence of anthraquinone pigments. Its colour contrast between brownish-red to brownish-black apothecial disc and yellowish-orange apothecial margin is very characteristic. Further diagnostic characters are a hymenium with numerous oil-drops and specific ecology. The species is related to *Caloplaca flavorubescens* and *C. flavovirescens*.

Key words — anthraquinones, ITS nrDNA, phylogeny, taxonomy, *Teloschistaceae*

Introduction

A study of specimens of the genus *Caloplaca* Th. Fr. collected during field trips in the mountains of South Korea by L. Lőkös and Y. Joshi, revealed an interesting and unusual saxicolous species, which is described here as new to science. The new species has been found only at inland localities in three mountains, Mt Jobong, Mt Taebaek, and Mt Gyeokja. It grows in open woodlands on siliceous and non-calcareous rocks together with abundant members of *Aspicilia*, *Cladonia*, *Heterodermia*, *Lecanora*, *Leptogium*, *Myelochroa*, *Pertusaria*, *Phaeophyscia*, *Porpidia,* and *Ramalina*.

Although we obtained only three specimens of this new taxon, we describe it without any hesitation because 1) the species is morphologically well defined,

2) one may wait for too a long time to obtain more material of this rare taxon when conservationists and scientists could benefit from the data, 3) DNA evidence supports our hypothesis, and 4) the material was collected in ample amount and duplicates sent to several herbaria.

Materials & methods

Studied specimens are deposited in CBFS, KH, KoLRI, UCR and the private herbarium of J. Halda (Czech Republic). Description and photographs of external morphology are based on air-dried material observed under a dissecting stereomicroscope (Nikon SMZ645). Sections were made with a razor blade under the stereomicroscope and mounted in lactophenol cotton blue. Anatomical descriptions are based on these preparations under a compound microscope (Nikon Eclipse E200). The mycological terminology follows Kirk et al. (2008). Measurements of ascospores were made at ×400 magnification mounted in water; only free ascospores lying outside the asci were measured. The results are recorded as 95% confidence interval of the measured values. Number of measurements (n) is within square brackets. Chemical constituents were identified by HPLC (Søchting 1997).

DNA extraction and amplification. Direct PCR was used for PCR-amplification of the ITS regions including the 5.8S gene of the nuclear rDNA following Arup (2006). Primers for amplification were ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). PCR cycling parameters follow Ekman (2001).

Phylogenetic analysis. Five newly obtained ITS sequences were included in the phylogenetic analysis along with six sequences from GenBank (Tab. 1) to illustrate the phylogenetic position of *Caloplaca allochroa* within the *Caloplaca flavorubescens*/*C. flavovirescens* clade. *Caloplaca crenularia* and *C. ferruginea* were selected as outgroup. Sequences were aligned using BioEdit and manually cut to eliminate the unaligned

Table 1. Sequences used in phylogenetic analysis.*

Species	Voucher data	Genbank accession number
Caloplaca allochroa Y. Joshi, Vondrák & Hur	South Korea; CBFS JV7987	**HQ415800**
C. crenularia (With.) J.R. Laundon	Arup et al. 2007	EF643512
C. egeana Cl. Roux & Nav.-Ros.	Gibraltar; CBFS JV6262	**HQ644198**
C. ferruginea (Huds.) Th. Fr.	Arup & Åkelius 2009	FJ866808
C. flavorubescens (Huds.) J.R. Laundon	Wei et al. 2008	EU266111
	Wedin et al. 2002	AY143394
	Kasalicky et al. 2000	AF279887
C. flavovirescens (Wulfen) Dalla Torre & Sarnth.	Arup & Grube 1999	AF353966
C. juniperina Tomin	Tajikistan; CBFS JV7397	**HQ644200**
	Tajikistan; herb. Halda 174	**HQ644199**
C. stantonii W.A. Weber ex Arup	California; UCR 55976	**HQ644197**

* Newly obtained sequences shown in bold.

ends and ambiguously aligned regions of ITS1 and ITS2; 539 positions were retained. Bayesian phylogenetic analysis was carried out using the program MrBayes 3.1.1 (Ronquist & Huelsenbeck 2003). The optimal nucleotide substitution model (GTR+G) was found using the program MrModeltest v2.3 (Nylander 2004) with the Akaike Information Criterion and the hierarchical likelihood ratio test (Posada & Crandall 1998). The MCMC analysis was run for one million generations, performed in two runs, each with four chains starting from a random tree and using the default temperature of 0.2. Every 100th tree was sampled, and the first 215,000 generations were discarded as burn-in, using standard deviation of splits between runs less than 0.01 as a convergence criterion.

New species

Caloplaca allochroa Y. Joshi, Vondrák & Hur, **sp. nov.** FIG. 1

MycoBank MB 561554

Thallus crustaceus, continuus ad rimosus ad disperso-areolatus, laevigatus, tenuis, luteus vel cinereus, UV–. Prothallus praesentia, nigrescens. Apothecia biatorina, sessilia, rotundata ad angulatus, 0.3–0.8 mm. Discus planiusculus ad ±convexus, porphyreus ad fuscus, ±pruinosus. Proper margine flavoaurantiacus, persistens. Hymenium inspersum. Hypothecium indistinctus cellularis. Proper margine tenuis, prosoplectenchymatous. Thalline margine absentia. Paraphyses simplices, ±furcatus ad apicis, septatus. Asci 8-spori, ascosporae polarilocularis, ellipsoideus ad late ellipsoideus, 15–20 µm longae et 7.5–10 µm latae.

Type: South Korea. Gangwon Prov.: Yangyang-gun, Seo-myeon, Hwang-ri, Mt Jobong, 37°56′10″N, 128°33′74″E, alt. 980 m, on siliceous rock, 14 May 2009, Y. Joshi, X.Y. Wang, J.A. Ryu, J.Y. Hur 090296 (**holotype** KoLRI; **isotypes**, CBFS JV7987, KH).

Etymology — The species epithet derives from the Latin word *allochrous,* which means changing colour.

Thallus saxicolous, crustose, continuous to rimose to dispersed areolate, smooth, indeterminate, effuse, 6–8 cm in diam., 70–90 µm thick [n = 20], whitish-gray (Fig. 1A) or yellow (Fig. 1B). Cortex thin to moderately thick, 8–23 µm thick [n = 20], paraplectenchymatous, necral layer absent. Algal layer continuous, 25–50 µm high [n = 20]. Medulla hyaline, of densely interwoven hyphae, without crystals, 12.5–40 µm high [n = 20]. Prothallus blackish-gray.

Apothecia biatorine, numerous, scattered to ±aggregated, 0.3–1.0 mm in diam. [n = 20], sessile to ±constricted at the base, slightly concave when young, later plane to slightly convex; disc rounded to irregular, brownish-red to brownish-black, ± greenish-yellow pruinose (Fig. 1B); margin egg yolk coloured to yellowish-orange (distinctly paler than disc) to orange-brown, variously thick, flush, smooth to ±wavy, entire, persistent. Epihymenium dark golden brown, with numerous yellowish brown granules, 12.5–20 µm high [n = 20]. Hymenium hyaline, with numerous oil droplets not dissolving in K, 87.5–100 µm high [n = 20]. Hypothecium hyaline, of indistinct

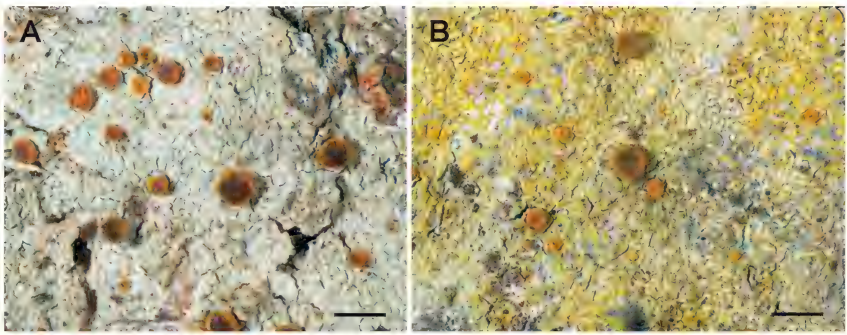

FIG. 1 *Caloplaca allochroa* (isotype). **A.** White-gray thallus with apothecia (Scale = 1 mm); **B.** Yellow thallus with apothecia (Scale = 1 mm).

cells, 75–110 µm [n = 20]. PROPER MARGIN thin to thick, 25–75 µm wide [n = 20], prosoplectenchymatous, with outer region yellowish-brown pigmented. Algal layer ±continuous below the hypothecium. THALLINE MARGIN absent. PARAPHYSES hyaline, simple to ±furcated at the apices, septate, 1.5–2 µm thick, tips 2.5–3 µm broad [n = 20]. ASCOSPORES 8 per ascus, hyaline, polarilocular, ellipsoid to broadly ellipsoid, 12.5–21.5 × 6–11.5 µm, isthmus 2.5–7.5 µm [n = 20]. PYCNIDIA rarely present, ostioles reddish-orange; conidia bacilliform, 2.1–2.5 × 0.7–0.9 µm [n = 20].

CHEMISTRY — Spot test reactions: thallus K– (grayish regions) or K+ purple (yellowish regions), C–, KC–, P–, UV–. Epihymenium K+ purple, C–. Apothecia contain parietin and fragilin (major anthraquinones), emodin and 7-chloroemodin (traces); fragilin was observed as the major pigment in yellow thalli together with traces of parietin, emodin and 7-chloroemodin. Only traces of parietin and fragilin were observed in white thalli. No acetone insoluble pigments were identified in darkened apothecial discs.

PHYLOGENY — *Caloplaca allochroa* is placed in the clade containing well-known species *C. flavorubescens* (Huds.) J.R. Laundon and *C. flavovirescens* (Wulfen) Dalla Torre & Sarnth. (PP=1.00). The clade is not internally well-resolved with *C. egeana*, *C. flavovirescens* and *C. stantonii* in the basal polytomy (FIG. 2).

ECOLOGY AND DISTRIBUTION — *Caloplaca allochroa* is so far known only from three mountains (Mt Jobong, Mt Taebaek, and Mt Gyeokja) in South Korea. The species occurs on non-calcareous rocks in subtropical inland areas at elevations from 350–980 m. It grows both on subvertical and horizontal faces of the rocks, preferring situations with sufficiency of diffuse sunlight, under open canopied stands. Both yellow and gray-white morphotypes are reported from Mt Jobong and Mt Gyeokja, while in Mt Taebaek only the gray-white morphotype has been collected.

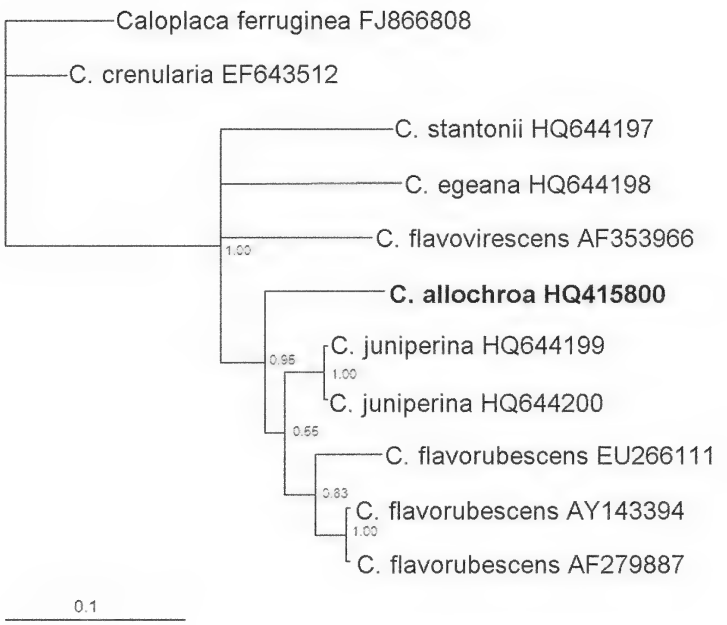

Fig. 2 Bayesian phylogenetic tree based on ITS nrDNA sequences showing the alliance of *Caloplaca allochroa* with *C. flavorubescens* and *C. flavovirescens*. Posterior probabilities are present at nodes.

Its three known communities have rather different lichen compositions. At the type locality *Caloplaca allochroa* was growing in a community with species of *Cladonia, Heterodermia, Leptogium, Myelochroa, Pertusaria, Porpidia* and *Ramalina*. The new species was accompanied only by *Aspicilia* sp. on Mt Taebaek but by *Leptogium, Phaeophyscia, Myelochroa, Pertusaria,* and *Lecanora* on Mt Gyeokja. Even though phorophytes, such as *Acer, Fraxinus, Rhododendron, Pinus,* and *Quercus* occur in all three *C. allochroa* localities, the new taxon has not yet been found growing on bark.

Additional specimens examined: **SOUTH KOREA. Gangwon Prov.**: Taebaek-si, Mungoksodo-dong, Mt Taebaek, 37°06′08.3″N, 128°57′05.4″E, alt. 970 m, on rock, 14 October 2005, L. Lőkös 050734 (KoLRI). **Jeollanam Prov.**: Wando Co., Bogil-myeon, Bogil Island, Buyong-ri, Mt Gyeokja, en route from Keungiljae to Suribong, 34°08′50″N, 126°32′90″E, alt. 368 m, on rock, 05 February 2010, Y. Joshi, H. S. Jeon & M . H. Jeong 100152 (KoLRI).

Remarks — This is a distinctive species characterized by the colour variation shown by its thallus (yellow to gray), dark brown-red biatorine apothecia with yellow-orange margin, and inspersed hymenium. The colour of the areoles is

yellow and the discs are orange brown without traces of black colour in less sun-exposed sites but on the strongly exposed rock faces, the thallus is distinctly gray and the apothecia are brownish-black.

The yellow morphotype of the new species is most likely to be confused with the saxicolous *Caloplaca flavovirescens* or the corticolous *C. alnetorum* Giralt et al., *C. flavorubescens,* and *C. gordejevii* (Tomin) Oxner ex Khodos., but none of the latter possesses such a strong colour contrast between apothecial disc and margin. Gray-white thallus morphotypes are similar to saxicolous *Caloplaca egeana* Cl. Roux & Nav.-Ros. and *C. subochracea* (Wedd.) Werner, and corticolous *C. aegatica* Giralt et al., which also differ in apothecial coloration. *Caloplaca lypera* Poelt & Hinter., a similar species from temperate or sub-alpine Himalayan regions, cannot be confused with this taxon, as it differs in having yellowish medulla and ochraceous-gray thallus (Poelt & Hinteregger 1993; Joshi & Upreti 2011). The combination of parietin and fragilin as main anthraquinones in the new species is rare within *Caloplaca*; it is known (as in *C. flavovirescens* and *C. flavorubescens;* Santesson 1970) and described as chemosyndrome B1 by Søchting (2001).

Existence of two colour thallus variants based on presence vs. absence of anthraquinones is probably not exceptional in *Teloschistaceae*; it has been recently described in *Caloplaca phlogina* (Ach.) Flagey (Vondrák et al. 2010) and also occurs in the North American *C. stanfordensis* H. Magn. (Vondrák, unpublished data).

Acknowledgments

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Four anamorphic fungi (with two new species) from forests of Western Ghats, India

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Abstract — *Anaselenosporella indica* and *Arachnophora goanensis*, two new species of anamorphic fungi isolated from decaying plant litter collected from the forest of Goa, India, are described and illustrated. *Anaselenosporella indica,* found growing on dead twig of an unidentified plant, is characterized by polyblastic, sympodial, discrete, conidiogenous cells and cylindrical, rarely curved, aseptate, hyaline conidia. *Arachnophora goanensis*, collected from dry decaying bark of an unidentified tree, is characterized by blastic pigmented stauroconidia and blastic hyaline fusiform synanamorphic conidia. Two other species from the monotypic genera *Catenosynnema* and *Cheiromyceopsis* are also reported for the first time from India.

Key words — biodiversity, taxonomy

During research on microfungi from forests of Western Ghats in Goa, four interesting fungi belonging to the anamorphic genera *Anaselenosporella* Heredia et al., *Arachnophora* Hennebert, *Catenosynnema* Kodsueb et al. and *Cheiromyceopsis* Mercado & J. Mena were isolated from fallen and decaying plant litter. Two are described and illustrated as new species, *Anaselenosporella indica* and *Arachnophora goanensis*. Collections of new species are conserved in the herbarium of Indian Agricultural Research Institute, New Delhi (HCIO) and new records at Department of Botany, Goa University, Goa.

Anaselenosporella indica Pratibha, Bhat & Raghuk. **sp. nov.** Figs 1, 2

MycoBank MB561104

Ad fungus anamorphicus. Conidiophora macronemata, mononemata, singula, erecta, laevia, recta vel leviter flexuosa, ramosa ad apicum, atrobrunnea ad basim, pallid brunnea ad apicum, multiseptata, 85–140 × 4.5–8 µm. Cellulae conidiogenae polyblasticae, lageniformes, leviter geniculatus et elongatus versus apicum, 15–30 × 2–4

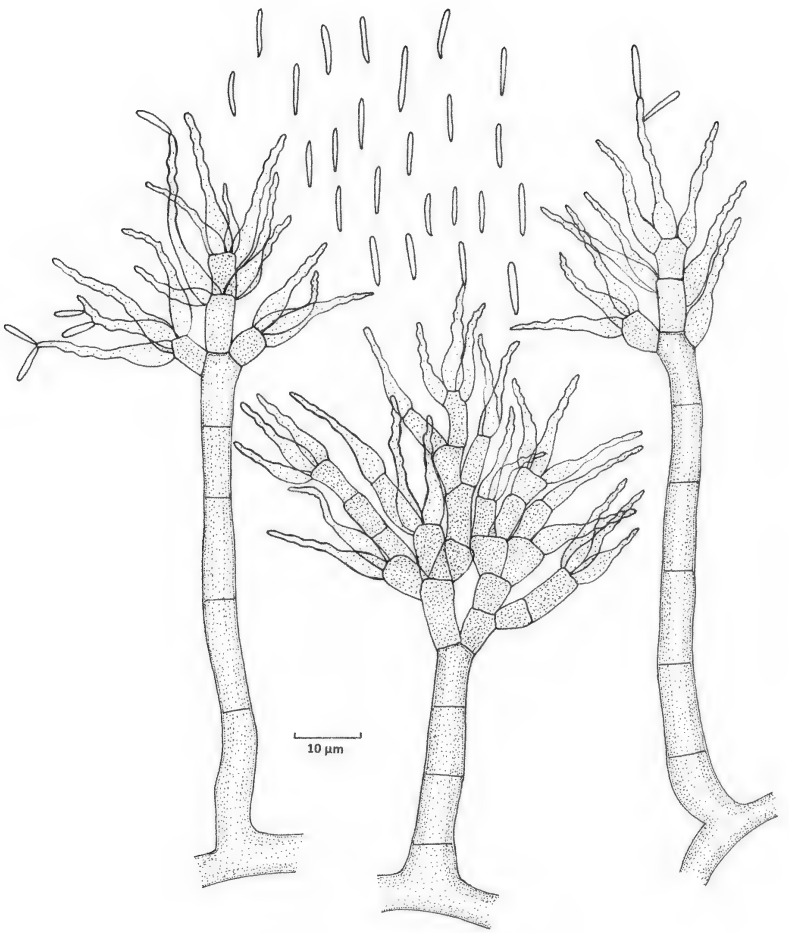

Fig. 1. *Anaselenosporella indica*.
Conidiophores, conidiogenous cells and conidia.

µm, indeterminatus, sympodialis, discretae ex ramis metuloideis, cuneiformibus, 10–14× 3–6 µm. Conidia solitaria, cylindrica, raro curvus, aseptata, utrinque rotandata, hyalina, laevia, 8–12 × 1.5 µm.

Type: India, Goa, Ponda, Bondla, on dead twig of unidentified tree, 20/08/2010, Pratibha J., **holotype:** HCIO 50176.

Etymology: *indicus* (Latin), referring to the country where the type was collected.

Anamorphic fungus. Colonies on natural substrate effuse, brown; mycelium partly superficial, partly immersed, composed of smooth, light brown, branched, septate, 2–3.5 µm wide hyphae. Colonies on PDA dark green,

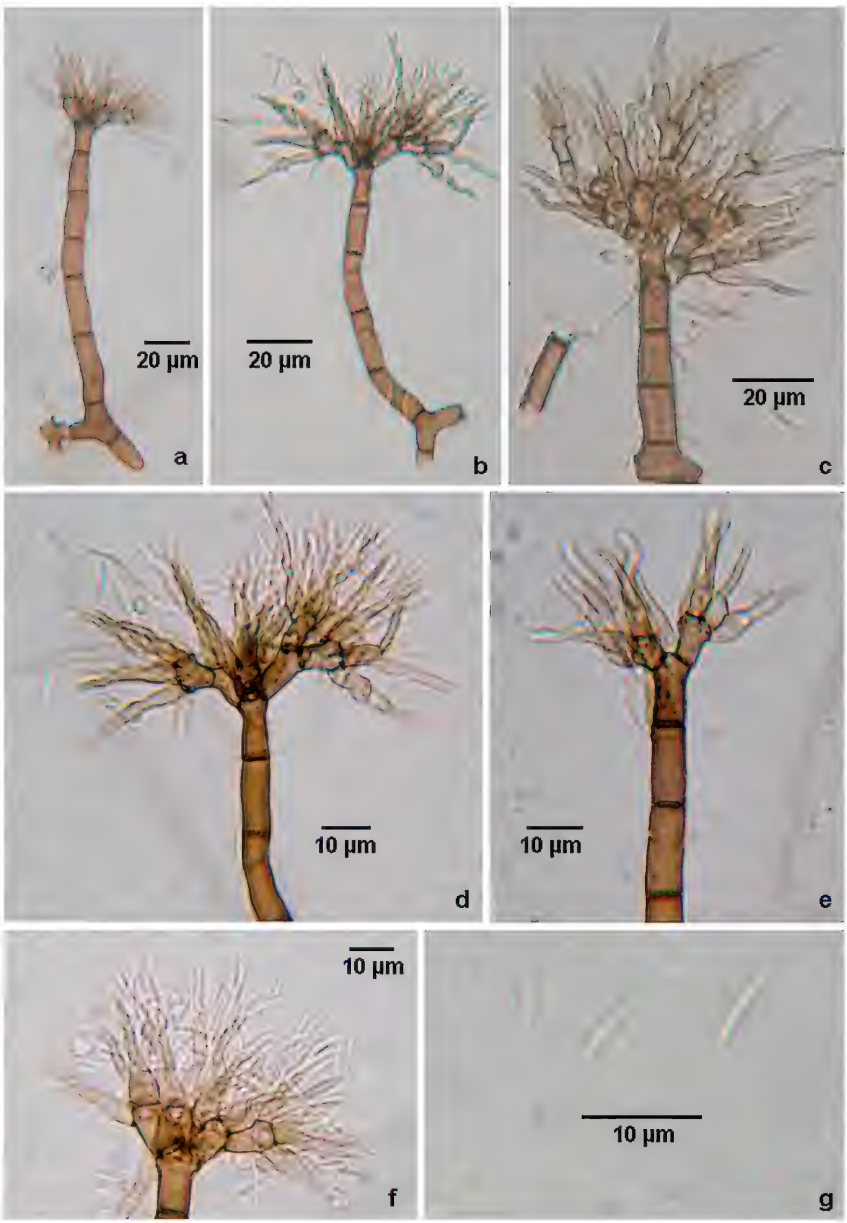

Fig. 2. *Anaselenosporella indica:* a–e. conidiophores, conidiogenous cells, and conidia; f. conidiogenous cells and conidia; g. conidia.

wooly, reverse black, margin serrated, attaining a diam. of 1.8 cm in 10 days. Conidiophores macronematous, mononematous, single, erect, smooth, straight to slightly flexuous, branched at the apex, dark brown at the base, pale brown towards the tip, multiseptate, 85–140 × 4.5–8 µm. Conidiogenous cells polyblastic, lageniform, slightly geniculate and elongated towards the apex, 15–30 × 2–4 µm, indeterminate, sympodial, discrete, formed in a compact cluster on cuneiform, dark brown, smooth, thick-walled, 10–14 × 3–6 µm metuloid branches. Conidia solitary, cylindrical, rarely curved, aseptate, rounded at both ends, hyaline, smooth, 8–12 × 1–1.5 µm.

Notes: Heredia et al. (Castañeda et al. 2010) established the monotypic genus *Anaselenosporella* with *A. sylvatica* as type species to accommodate a fungus with polyblastic, discrete, lageniform, sympodial conidiogenous cells formed on metuloid cells with acicular, filiform, fusiform to semi-circular, unicellular, hyaline conidia. *Anaselenosporella indica* differs from the type species in conidiophore and conidiogenous cell dimensions and conidiophore branching. In *A. sylvatica* the conidiophores are much larger (700–1200 × 12–28 µm) and dichotomously branched and the conidiogenous cells smaller (5–10 × 2–2.5 µm) than for *A. indica*. The type species is further differentiated by conidia that are acicular, curved to semicircular, truncate at the base, and not solitary, cylindrical, rarely curved, rounded at both ends as in the new species.

Arachnophora goanensis Pratibha, Bhat & Raghuk. **sp. nov.** Figs 3, 4

MycoBank MB561105

Ad fungos anamorphicus. Conidiophora macronemata, mononemata, apicem versus attenuata, singula, erecta, laevia, recta vel leviter flexuosa, non-ramosa, atrobrunnea ad basim, pallide brunnea ad apicem, multiseptata, 80–200 × 4.5–7 µm. Cellulae conidiogenae monoblasticae, cylindricae, terminales, integratae, determinatae vel interdum percurrentes, pallide brunneae, 5–8 × 2.5–4 µm. Conidia solitaria, blastica, acrogena, staurosporia, septata, sicca, laevia, 25–40 × 20–25 µm, cum unicus, atrobrunnea, 6–9 × 5.5–8.5 µm cellulae basali et 1-3, atrobrunneae, 3–7.5 × 5.5–8.5 µm cellulae centrales, edens ortus ad pallide brunnae cellulae laterales, ortus ad 1–4, hyalina ad pallide brunnea, 4–8 × 1–3 µm brachiis. Synanamorpha conidia hyalina, fusiformia, aseptata, 5–8 × 1 µm, in cellulae peripherales, oriunda.

Type: India, Goa, Sanguem, Netravali, on dry and decaying bark of unidentified tree, 28/09/10, Pratibha J., **holotype:** HCIO 50177.

Etymology: *goanensis* (Latin), referring to the state where the type was collected.

Anamorphic fungus. Colonies on natural substrate effuse, brown; mycelium partly superficial, partly immersed, composed of smooth, light brown, branched, septate, 2–3 µm wide hyphae. Colonies on PDA dark green, flat, reverse black, margin serrated, attaining a diam. of 1.4 cm in 10 days. Conidiophores differentiated, mononematous, tapering towards apex, single, erect, smooth, straight to slightly flexuous, unbranched, dark brown at the base,

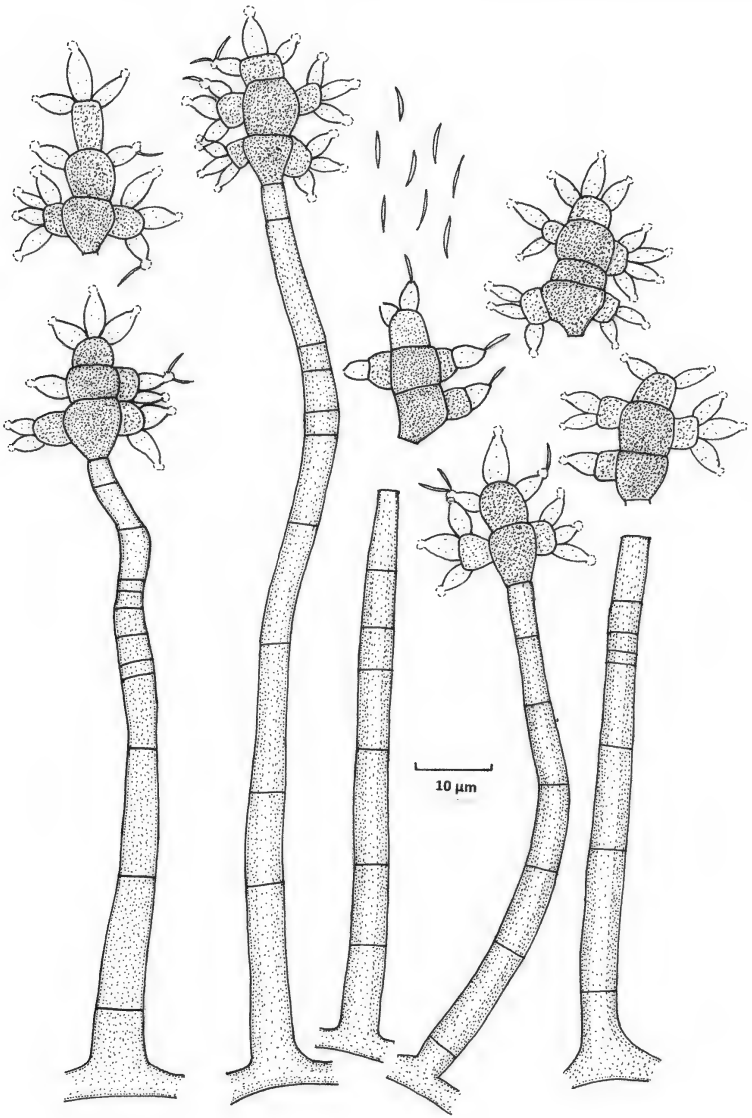

Fig. 3. *Arachnophora goanensis.*
Conidiophores, conidiogenous cells, stauroconidia, and *Selenosporella*-like synanamorph.

pale brown towards the apex, multiseptate, 80–200 × 4.5–7 µm. Conidiogenous cells monoblastic, cylindrical, terminal, integrated, determinate or sometimes percurrently proliferating, light brown, 5–8 × 2.5–4 µm. Conidia solitary,

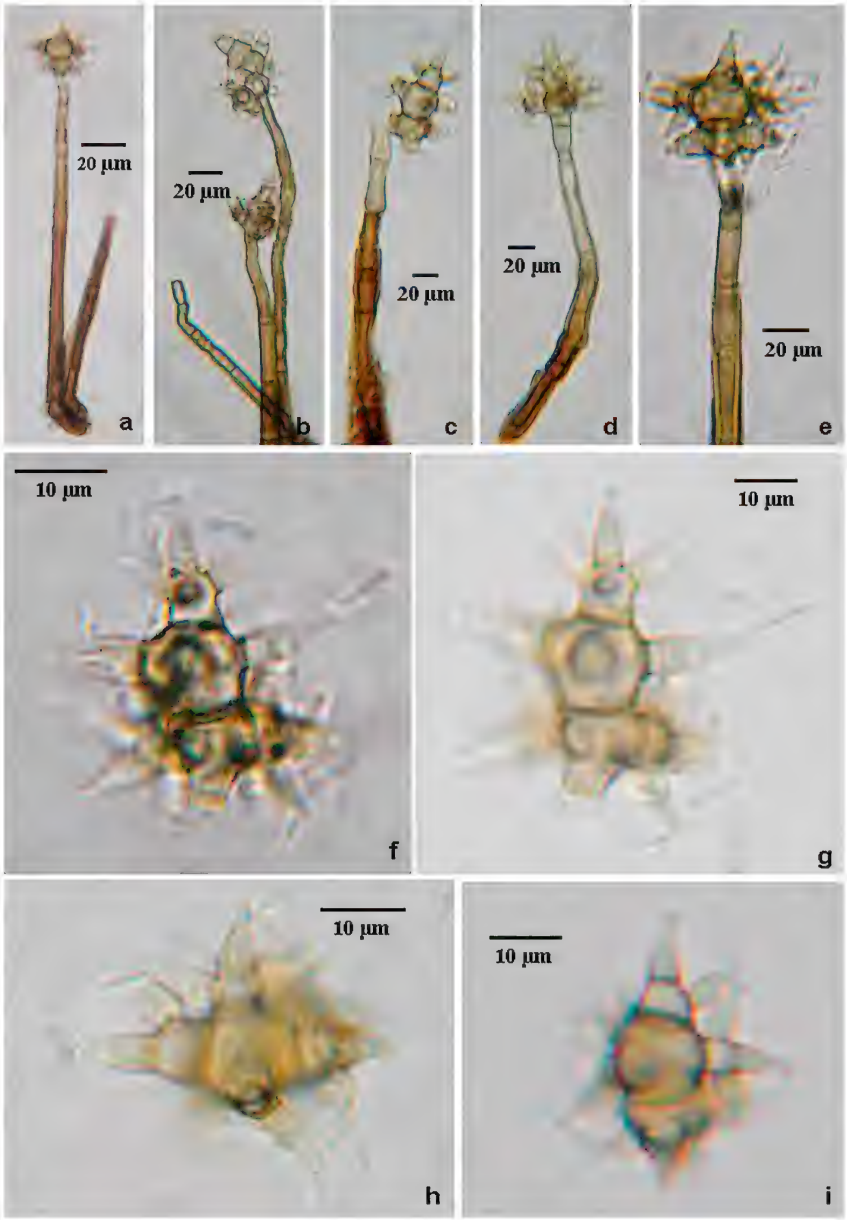

FIG. 4. *Arachnophora goanensis:* a–e. conidiophores, conidiogenous cells and conidia; f–i. stauroconidia, with *Selenosporella*-like synanamorph.

blastic, acrogenous, staurosporous, septate, dry, smooth, 25–40 × 20–25 µm, with one, dark brown, 6–9 × 5.5–8.5 µm basal cell and 1–3, dark brown, 3–7.5 × 5.5–8.5 µm central cells; each central cell either gives rise directly to 2 or more fertile, hyaline to pale brown arm-cells, or to a light brown lateral cell, which in turn gives rise to 1–4 fertile arms; fertile arms hyaline to pale brown, 4–8 × 1–3 µm, producing synanamorphic conidia at the tip. *Selenosporella*-like synanamorphic conidia hyaline, fusiform, aseptate, 5–8 × 1 µm, formed on tip of the peripheral cells of each conidial arm.

Notes: Hennebert (1963) established *Arachnophora* to accommodate a fungus having monoblastic, terminal, integrated, percurrently proliferating conidiogenous cells and staurosporous, solitary, pigmented, acrogenous conidia and with usually two central cells in the axis and several radial, recurved, conical arms that arise from the central cells. Of the eight species previously included in the genus, only six are currently accepted (Table 1): *Arachnophora crassa*, *A. excentrica* (B. Sutton) S. Hughes [≡ *Digitoramispora excentrica* (B. Sutton) R.F. Castañeda & W.B. Kendr.], *A. fagicola* (type species), *A. hughesii, A. polyradiata, A. pulneyensis, A. simplex* Ichinoe [≡ *Uberispora simplex* (Ichinoe) Piroz. & Hodges], and *A. uberisporoides* (Hennebert 1963, Pirozynski & Hodges 1973, Révay & Gönczöl 1989, Castañeda & Kendrick 1990, Castañeda et al. 1996, 1997; Castañeda & Guarro 1998).

Table 1: Synopsis of accepted species of *Arachnophora*.

Species	Conidia	*Selenosporella*-like synanamorph
A. crassa Révay & Gönczöl	2-celled central body, 16–22 × 8–9.6 µm, each cell bearing 1–2-celled lateral branches	Borne directly on the apex of lateral branches
A. fagicola Hennebert	2-celled central body, 16–20 × 10–13 µm, each cell bearing several lateral protuberances, each bearing 1 or more inwardly curved, claw- or spine-like processes	Absent
A. goanensis	2–4-celled central body, 25–40 × 20–25 µm; each central cell bearing aseptate, hyaline to pale brown arms, 4–8 × 1–3 µm, borne directly on either central cell or light brown lateral cells	Conidia blastic, hyaline, fusiform, aseptate, 5–8 × 1 µm
A. hughesii R.F. Castañeda & Guarro	2–3-celled central body, 27–30(–37) × 17–24(–30) µm, with 2–5 tapered, 0–1-septate horn-like or conical arms	Conidia blastic, fusiform, aseptate, colourless, 3–4 × 0.5–1 µm
A. polyradiata (Mercado & R.F. Castañeda) R.F. Castañeda & W. Gams	3-celled central body, 28–40 × 25–38 µm, each cell bearing 2–3-septate, radiating arms	Conidia hyaline, aseptate, 4.5–7 × 0.5–1 µm
A. pulneyensis (Subram. & Bhat) R.F. Castañeda	2–3-celled central body, 16–24 × 6.4–8.1 µm, with up to 4 arms	Absent
A. uberisporoides R.F. Castañeda	2-celled central body, 17–20 × 9–13 µm, with 4–6 dome-shaped arms	Absent

Arachnophora goanensis resembles *A. polyradiata* in conidial shape and the *Selenosporella*-like synanamorph at the tip of the arms with fusiform, hyaline, 1-celled conidia. However, it differs in conidiophore dimension and number of conidial cells and arms. The *A. polyradiata* conidiophores are shorter (≤ 50 µm long) and the conidia are composed of two dark brown central cells, with each cell giving rise to one 2–3-septate, radiating arm.

Catenosynnema micheliae Kodsueb et al. Cryptog. Mycol. 28: 239. 2007. Figs 5a, b

Anamorphic fungus. Conidiomata sporodochial, superficial, light brown. Conidiophores branched, light brown, 40–70 × 2–4 µm. Conidiogenous cells blastic, integrated, determinate, sub-hyaline. Conidia in simple to branched acropetal chains, aseptate, light brown, smooth, ellipsoidal to fusiform, 8–12 × 3–5 µm.

Specimen examined: **INDIA. Maharashtra:** Amboli, on dead and decaying twigs of unidentified tree, 18/08/09, Pratibha J., GUBH MT01.

Cheiromyceopsis echinulata Mercado & J. Mena, Acta Bot. Cubana 53: 2. 1988. Figs. 5c, d

Anamorphic fungus. Conidiomata sporodochial, superficial, light brown. Conidiophores macronematous, brown, 20–40 × 5–7 µm. Conidiogenous cells monoblastic, integrated, terminal, determinate. Conidia cheiroid, dark brown, 22–30 µm long, branched; branches 3–7, verrucose, septate, 15–20 x 5–7.5 µm.

Specimen examined: **INDIA. Karnataka:** Uttar Kannada, Kathlekan, on dry and decaying bark of unidentified tree, 17/11/09, Ashish P., GUBH MT02.

Discussion

This paper is the first report of the genera *Anaselenosporella, Catenosynnema,* and *Cheiromyceopsis* from India, and of the genus *Arachnophora* from the Western Ghats. The genus *Anaselenosporella* was monotypic, reported only from Mexico. *Catenosynnema* and *Cheiromyceopsis* are monotypic, previously reported from Cuba and Thailand, respectively. The genus *Arachnophora* is widely distributed. The collections from Western Ghats form an interesting observation.

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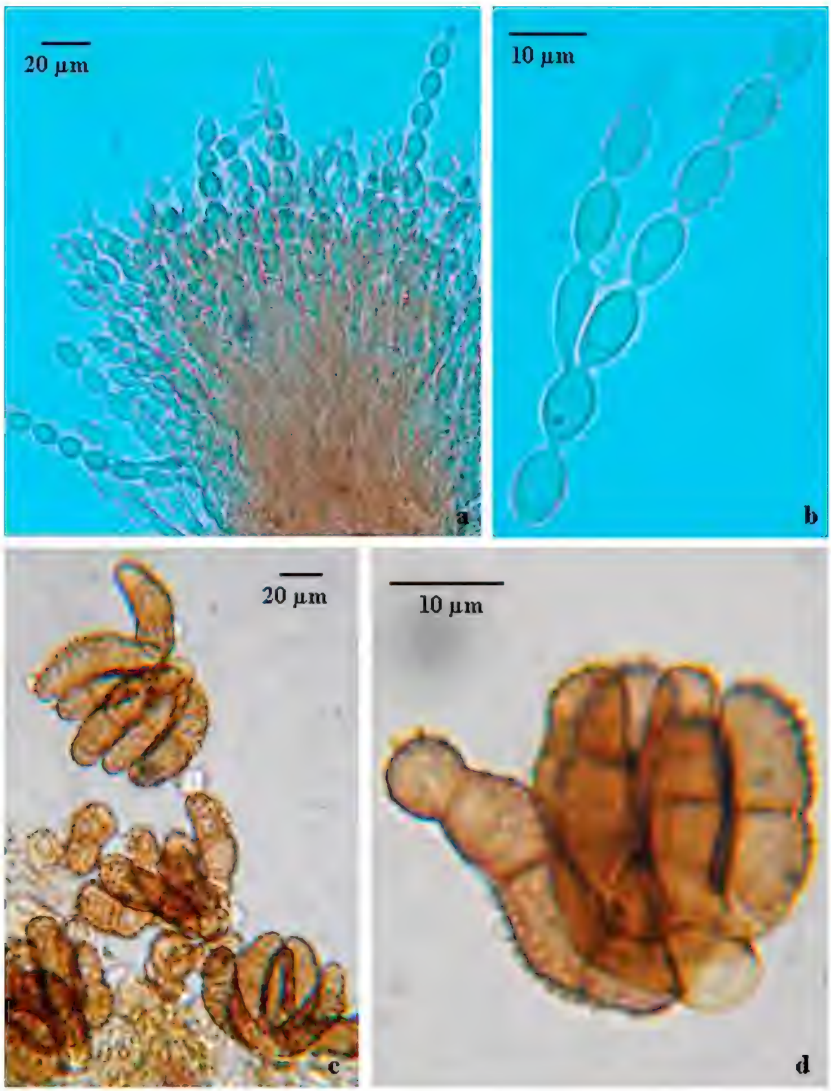

Fig. 5. a–b: Sporodochia and conidial chain of *Catenosynnema micheliae.*
c–d: Cheiroid conidia of *Cheiromyceopsis echinulata.*

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Taxonomic studies of *Endophragmiella* from southern China

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Abstract — Two new species, *Endophragmiella clausenae* from dead branches of *Clausena lansium* and *E. pentaphylacis* from dead branches of *Pentaphylax euryoides*, are described and illustrated. *Endophragmiella corticola* is a new record for China. These three species were collected from tropical and subtropical forests in southern China.

Key words — hyphomycetes, taxonomy

Introduction

Sutton (1973) established *Endophragmiella* B. Sutton to accommodate two species, *E. pallescens* B. Sutton and *E. canadensis* (Ellis & Everh.) B. Sutton. The genus is characterized by macronematous, branched or unbranched conidiophores with terminal, integrated, percurrently proliferating and monoblastic conidiogenous cells producing solitary and euseptate or distoseptate conidia with rhexolytic secession (Sutton 1973, Ellis 1976, Hughes 1979, Wu & Zhuang 2005). These characters separate *Endophragmiella* from similar genera, *Phragmocephala* E.W. Mason & S. Hughes, *Chaetendophragmia* Matsush., and *Melanocephala* S. Hughes. Of the 80 species now assigned to *Endophragmiella*, most are saprobes, occurring on rotten wood, dead branches, and decaying leaves (Wu & Zhuang 2005).

Fungal diversity in southern China is high, and many wood-inhabiting fungi have been discovered (Dai et al. 2009, Dai and Li 2010, Ma et al. 2011). In our study on conidial fungi on dead wood from the tropical and subtropical forests in southeast China, two hitherto undescribed species and a new record of *Endophragmiella* were collected. The specimens are deposited in HSAUP (Herbarium of the Department of Plant Pathology, Shandong Agricultural University) and HMAS (Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences).

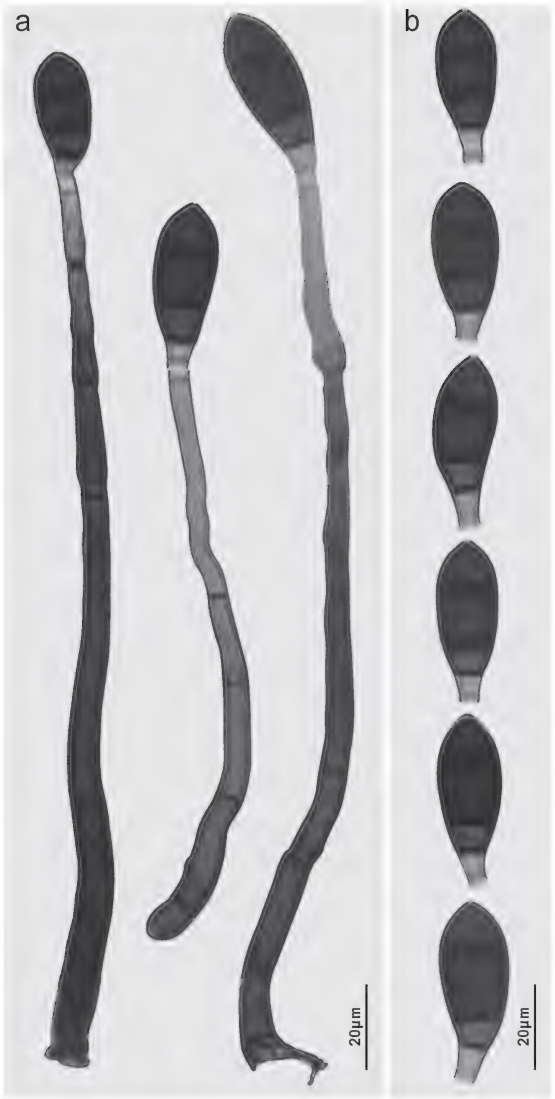

Fig. 1. *Endophragmiella clausenae*. a. Conidiophores and conidia. b. Conidia.

Taxonomy

Endophragmiella clausenae L.G. Ma & X.G. Zhang, **sp. nov.** Fig. 1

MycoBank MB 561597

Coloniae effusae, brunneae. Mycelium partim immersum, partim superficiale ex hyphis septatis, ramosis, laevibus, pallide brunneis, 2–4 μm latis, compositum. Conidiophora

solitaria, macronemata, mononemata, recta vel flexuosa, erecta, simplicia, multiseptata, laevia, brunnea, 135–410 × 4.5–8.5 µm. Cellulae conidiogenae *monoblasticae, terminals, in conidiophoris incorporatae, cum proliferationibus percurrentes, cylindricae, laeviae, pallide brunneae. Secessio conidiorum rhexolytica.* Conidia *solitaria, acrogena, 3-euseptata, ellipsoidea, clavata vel pyriformia, simplicia, sicca, laevia, brunnea vel atrobrunnea, cellula basalis 4.5–6 µm crassa, pallide brunneis, 31.5–42.5 ×14–16.5µm.*

Type: China, Yunnan Province: the Forbidden Forest of Banna, on dead branches of *Clausena lansium* (Lour.) Skeels (*Rutaceae*), 17 Oct. 2008, L.G. Ma (**holotype** HSAUP H0074; isotype HMAS 146108).

Etymology: in reference to the host genus, *Clausena*.

Colonies on the natural substrate effuse, brown. Mycelium partly immersed, partly superficial, composed of septate, branched, smooth-walled, pale brown hyphae, 2–4 µm thick. Conidiophores solitary, macronematous, mononematous, straight or flexuous, erect, simple, multiseptate, smooth, brown, 135–410 × 4.5–8.5 µm. Conidiogenous cells monoblastic, terminal, integrated, with conspicuous percurrent proliferations, cylindrical, smooth, pale brown. Conidial secession rhexolytic. Conidia solitary, acrogenous, 3-euseptate, ellipsoid, clavate to pyriform, simple, dry, smooth, brown to dark brown, basal cells 4.5–6 µm wide, pale brown, 31.5–42.5 × 14–16.5 µm.

Endophragmiella clausenae is similar to *E. bisbyi* (Hughes 1978a) and *E. mexicana* (Mercado et al. 1995) in conidial shape. *Endophragmiella bisbyi* differs from the new species by its smaller (9–13 × 5–6.5 µm) conidia with the thick distal septum and brown distal cell, while *E. mexicana* is easily distinguished by its much smaller (11–16 × 5.8–7.8 µm) predominantly 4-septate conidia with paler distal and basal cells.

Endophragmiella pentaphylacis L.G. Ma & X.G. Zhang, **sp. nov.** Fig. 2

MycoBank MB 561604

Coloniae effusae, brunneae. Mycelium partim immersum, partim superficiale ex hyphis septatis, ramosis, laevibus, subhyalinis vel pallide brunneis, compositum. Conidiophora *solitaria, macronemata, mononemata, recta, erecta, simplicia, septata, laevia, pallide brunnea vel brunnea, 56–92 × 4.5–7.5 µm.* Cellulae conidiogenae *monoblasticae, terminals, in conidiophoris incorporatae, cum proliferationibus percurrentes, cylindricae, laeviae, pallide brunneae. Secessio conidiorum rhexolytica.* Conidia *solitaria, acrogena, 3-septata, late ellipsoidea vel pyriformia, simplicia, sicca, laevia, brunnea vel atrobrunnea, cellula basalis 2.5–5 µm crassa, pallide brunneis, 25–32.5 × 13–16.5µm.*

Type: China, Yunnan Province: the Forbidden Forest of Banna, on dead branches of *Pentaphylax euryoides* Gardner & Champ. (*Pentaphylaceae*), 18 Oct. 2008, L.G. Ma (**holotype** HSAUP H0042; isotype HMAS 146109).

Etymology: in reference to the host genus, *Pentaphylax*.

Colonies on the natural substrate effuse, brown. Mycelium partly immersed, partly superficial, composed of septate, branched, smooth-walled, subhyaline to pale brown hyphae. Conidiophores solitary, macronematous,

Fig. 2. *Endophragmiella pentaphylacis*. a. Conidiophores and conidia. b. Conidia.

mononematous, straight, erect, simple, septate, smooth, pale brown to brown, 56–92 × 4.5–7.5 µm. Conidiogenous cells monoblastic, terminal, integrated, with conspicuous percurrent proliferations, cylindrical, smooth, pale brown. Conidial secession rhexolytic. Conidia solitary, acrogenous, 3-septate, broadly ellipsoid to pyriform, simple, dry, smooth, brown to dark brown, basal cells 2.5–5 µm wide, pale brown, 25–32.5 × 13–16.5 µm.

Endophragmiella pentaphylacis resembles *E. bisbyi* (Hughes 1978a), *E. ontariensis* (Hughes 1978b), *E. ellisii* (Hughes 1979), and *E. suttonii* (Kirk 1981) in conidial shape, but it can be differentiated from *E. suttonii*, *E. ontariensis* and *E. ellisii* by its larger, predominantly 3-septate and dark brown to black conidia. *E. bisbyi* can be separated from *E. pentaphylacis*, which has smaller (9–13 × 5–6.5 µm), versicolored conidia.

Endophragmiella corticola P.M. Kirk, Trans. Br. Mycol. Soc. 78(1): 60, 1982. Fig. 3

Colonies on the natural substrate pale brown to brown, effuse. Mycelium mostly superficial composed of branched, septate, subhyaline to pale brown, smooth-walled hyphae, 1.5–3 µm thick. Conidiophores macronematous,

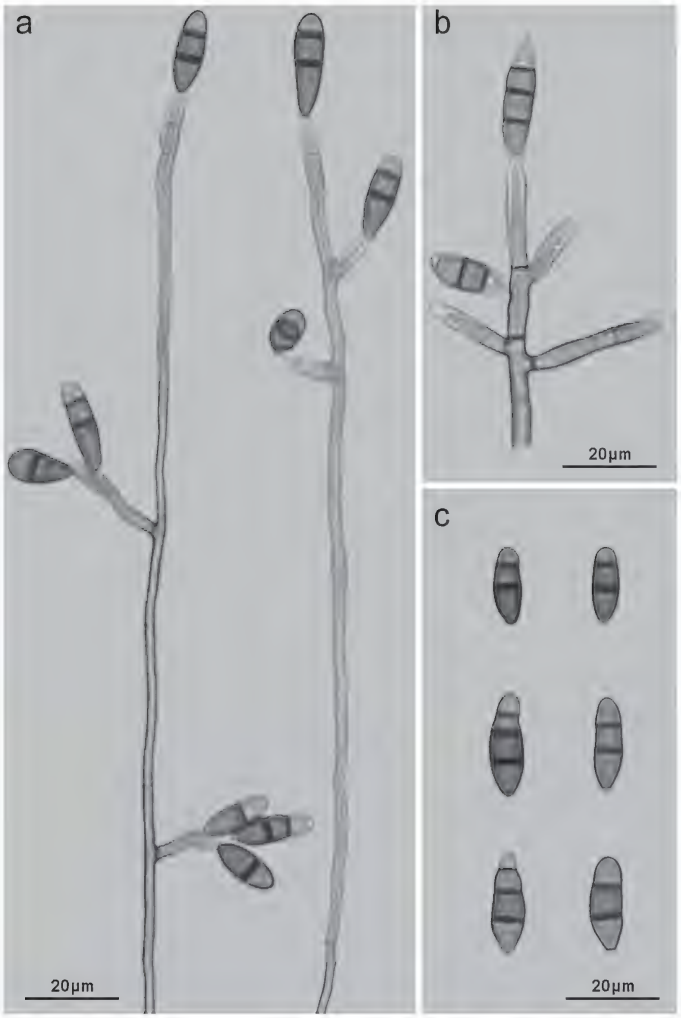

Fig. 3. *Endophragmiella corticola*. a–b. Branched conidiophores and conidia. c. Conidia.

mononematous, arising terminally and laterally from the mycelium, straight or flexuous, erect, branched, septate, smooth, pale brown, up to 350 μm high, 2.5–4 μm wide. Conidiogenous cells monoblastic, terminal, integrated, percurrent, cylindrical, tapered to a truncate apex, smooth, subhyaline. Conidial secession rhexolytic. Conidia solitary, acrogenous, 2–3-septate, narrowly obclavate to obclavate, simple, smooth, basal cells 1.5–3 μm wide, pale brown to brown, apical cells subhyaline, 16.5–25 × 5.5–7.5 μm.

Specimens examined: China, Fujian Province: the National Forest Park of Wuyishan, on dead branches of *Ficus gibbosa* Blume (*Moraceae*), 14 Aug. 2009, L.G. Ma, HSAUP H1026 (duplicate HMAS 146110).

Endophragmiella corticola is similar to *E. eboracensis* (Sutton 1975), *E. acuta* (Wu & Zhuang 2005), *E. verticillata* (Hughes 1978c), *E. curvata* (Hughes 1979), and *E. cesatii* (Hughes 1979). Ellipsoidal, shorter, 3-septate conidia and verticillately branched conidiophores distinguish *E. verticillata*, while *E. eboracensis* can be separated by its much shorter and mainly 3-septate conidia. *Endophragmiella acuta* has wider, 3-septate conidia with a tapered and rostrate apex and occasionally branched conidiophores; *E. curvata* has predominantly 2-septate conidia and unbranched conidiophores; and *E. cesatii* has wider, predominantly 3-septate conidia with brown central cells and unbranched conidiophores. The type material differs from the Chinese specimen in generally longer (14–42 μm) conidia and shorter (< 60 μm) conidiophores. This is the first record from China.

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Rhexodenticula zhengii sp. nov. from fallen leaves from China

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Abstract— A new species, *Rhexodenticula zhengii* from leaf litter of *Sycopsis sinensis* collected from Houhe National Nature Reserve, Wufeng, Hubei, China is described and illustrated with cymbiform conidia 18.5–22.0 × 4.5–5.0 µm, constricted at proximal and distal septa. A key to the species of *Rhexodenticula* is provided.

Key words— anamorphic fungi, rhexolytic secession, taxonomy

Introduction

Sycopsis sinensis is a native broadleaved evergreen woody plant species in China. It grows in mixed evergreen broadleaved forests or bushes and is sporadically distributed at elevations of 1000–2000 m in southern China (Li et al. 2003). However, the largest population of *S. sinensis* as the dominant species was reported in the Houhe National Nature Reserve by Liu et al. (1999).

A field trip was made to the Houhe National Nature Reserve (29°59′–30°10′N 110°22′–110°52′E) in Wufeng Tujia Autonomous County, Hubei, China to collect hyphomycetes in August 2008. A species of *Rhexodenticula* new to science was discovered on decaying leaves of *Sycopsis sinensis*.

Materials & methods

Conidiophores and conidia of the fungus were mounted in 85% lactic acid. Tape lifts were prepared for wet mounting using Mainstays Crystal Clear Tape (Imported by Walmart Canada, Mississauga, Ontario). Microscopic observations were made using bright field and Nomarski differential interference contrast optics. Photomicrographs were taken with an Olympus Microfire digital camera (Goleta, CA). Herbarium acronyms follow the Index Herbariorum (Holmgren & Holmgren 1998). Measurements of the fungal structures were statistically analyzed with Microsoft Office Excel 2007

with 95% confidence interval of means. The results were presented as ranges and mean ± standard deviation. Q is length/width ratio and n, the number of fungal structures measured.

Results

Rhexodenticula zhengii D.W. Li & Jing Y. Chen, **anam. sp. nov.** FIGS 1–6

MycoBank MB 561153.

Conidiophora distincta, determinata vel percurrenter et sympodialiter proliferentia, erecta, solitaria vel fasciculata, non-ramosa, laevia, brunnea, 28–132(-205) × (3.5–)3.5–4.5(–4.5) µm, 1–9-septata. Cellulae conidiogenae polyblasticae, terminales, integratae, denticulatae. Conidia solitaria, obclavata, 3-septata, brunnea, verruculosa, (13.0–)18.5–22.0 × (4.0–)4.5–5.0(–5.5) µm ad basim margine, protrudente praedita. Teleomorphosis ignota.

TYPE: China. Hubei: Wufeng, Houhe National Nature Reserve, superficie in folio emortuo *Sycopsis sinensis* Oliv. (*Hamamelidaceae*) Coll. xxviii-viii-2008, De-Wei Li, [BPI 881495, **holotype**].

ETYMOLOGY: in honor of Prof. Ruyong Zheng, a fellow of The Chinese Academy of Sciences, for her contributions to the systematics of powdery mildews and *Zygomycota*.

CONIDIOPHORES differentiated, single or in groups, determinate or with percurrent and sympodial extension, erect, unbranched, straight or flexuous, sometimes geniculate, , dark brown, smooth, 1–9-septate, varying in length, 30–135(–205) × (3.4–)3.5–4.5(–5) µm (mean = 79 ± 53, × 4 ± 0.5, n = 21), more or less uniform in width with enlarged subglobose basal cell and 0–3 nodes, thick-walled, wall 0.5–0.8 µm thick. CONIDIOGENOUS CELLS integrated, terminal, polyblastic, denticulate, (12.5–)16.5–30.0(–33.5) × (3.0–)3.5–4.0 (–4.5) (mean = 23.5 ± 6.5 × 3.7 ± 0.3, n = 20) µm; denticles (0.6–)0.8–1.2(–1.5) × 0.7–1.1(–1.5) (mean = 1 ± 0.2 × 0.9 ± 0.2, n = 17) µm. CONIDIA 3-septate, apical and lateral, single, obclavate, brown, verruculose, (13.0–)18.5–22.0 (mean = 20.1 ± 1.7, n = 30) × (4.0–)4.5–5.0(–5.5) (mean = 4.6 ± 0.3, n = 30) µm, Q = (4.0)4–5.0(5.5) (mean = 4.4 ± 0.4, n = 30), the middle cells darker and longer than the apical and basal cells, constricted at proximal septum and slightly constricted at distal septum, basal cell rounded with a narrow vestigial, marginal frill at the base derived from the rhexolytically split separating cell at the middle, the frill (0.5–)0.7–1.1(–1.2) × (0.8–)0.9–1.1(–1.3) (mean = 0.9 ± 0.2 × 1 ± 0.1, n = 30) µm.

TELEOMORPH: unknown.

DISTRIBUTION AND HABITAT: Known from Hubei, China on fallen leaves of *Sycopsis sinensis*.

Discussion

Rhexodenticula was established by Baker & Morgan-Jones (2001) and typified by *Rhexodenticula cylindrospora* (R.F. Castañeda et al.) W.A. Baker & Morgan-Jones. Segregation of *Rhexodenticula* from *Nakataea* was based on its key

Figs 1–6. *Rhexodenticula zhengii*. 1–2. Conidiophores and conidiogenous cells; arrows in 2 indicate separating cell walls. 3. Basal portions of conidiophores. 4–6. Conidia; the arrow in 5 indicates a separating cell wall. Scale bars: 1, 4–6 = 10 μm, 2–3 = 5 μm.

character of releasing conidia by rhexolytically splitting a separating cell. The genus previously had two species, *Rhexodenticula elegiae* Melnik et al. (Mel'nik et al. 2004) being the second. *Rhexodenticula zhengii* adds a third species. All three species have four-celled conidia. *Rhexodenticula cylindrospora* has cylindrical conidia with four cells of equal length (Castañeda et al. 1996, Baker et al. 2001), while conidia of *R. elegiae* and *R. zhengii* are much shorter with paler end cells. The conidia of *R. zhengii* are obclavate, larger, and constricted at basal and distal septa: these characters can differentiate it from *R. elegiae*, which has fusiform conidia with unconstricted septa.

Key to species of *Rhexodenticula*

1. Conidia cylindrical, 13–21 × 4–5 µm ..... *R. cylindrospora*
1a. Conidia obclavate or fusiform 2
2. Conidia constricted at proximal and distal septa, obclavate, 18.5–22 × 4.5–5 µm ..... *R. zhengii*
2a. Conidia not constricted at septa, fusiform, 16–19 × 5 µm ..... *R. elegiae*

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Septobasidium atalantiae sp. nov. (*Septobasidiaceae*) and *S. henningsii* new to China

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Abstract —A new species, *Septobasidium atalantiae* on *Atalantia buxifolia* associated with nymphal stage of a scale insect, and a new Chinese record of *Septobasidium henningsii* on *Memecylon floribundum* are reported. They were collected from Hainan Province, China.

Key words —*Pucciniomycetes*, *Septobasidiales*, taxonomy

In 2010 some *Septobasidium* specimens were collected by the second author and her colleagues from Hainan Province. Among them a new species of *Septobasidium* on *Atalantia buxifolia*, which is associated with the nymphal stage of a scale insect, is described as follows:

Septobasidium atalantiae S.Z. Chen & L. Guo, **sp. nov.** Figs. 1–7

MycoBank MB 561544

Basidiomata resupinata, 0.2–7 cm longa, 0.15–1.4 cm lata, alba vel cinnamomeo-brunnea, margine indeterminata, superficie laevia vel velutina, saepe protuberantia, interdum maturitate fissurata, in sectione 480–1200 µm crassa. Subiculum brunneum, 25–100 µm crassum. Columnae brunneae, 20–50 µm altae, 20–70 µm latae. Strata hypharum 420–830 µm alta. Ab strato hymenii hyphae saepe repullulantes tum stratum hypharum et hymenium secundum formantes. Hymenium hyalinum, 60–70 µm crassum. Basidia cylindrica, curvata, 4-cellularia, 43–53 × 7.5–8.5 µm, hyalina. Sterigmata cylindrica vel conica, 6–7 × 3–5 µm. Sine probasidio. Basidiosporae non visae. Haustoria ex hyphis irregulariter spiralibus vel parallelis constantia.

Type: On *Atalantia buxifolia* (Poir.) Oliv. (*Rutaceae*): China, Hainan Province, Haikou, alt. 420 m, 1.XII.2010, L. Guo 11536, HMAS 251151 (**holotype**), associated with nymphal stage of a scale insect.

Basidiomata on branches, spreading to leaves, resupinate, 0.2–7 cm long, 0.15–1.4 cm wide, forming small isolated or confluent patches, white or

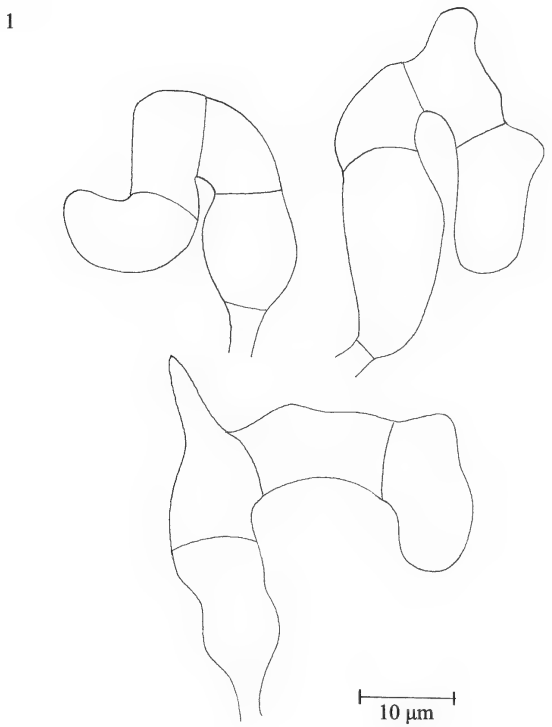
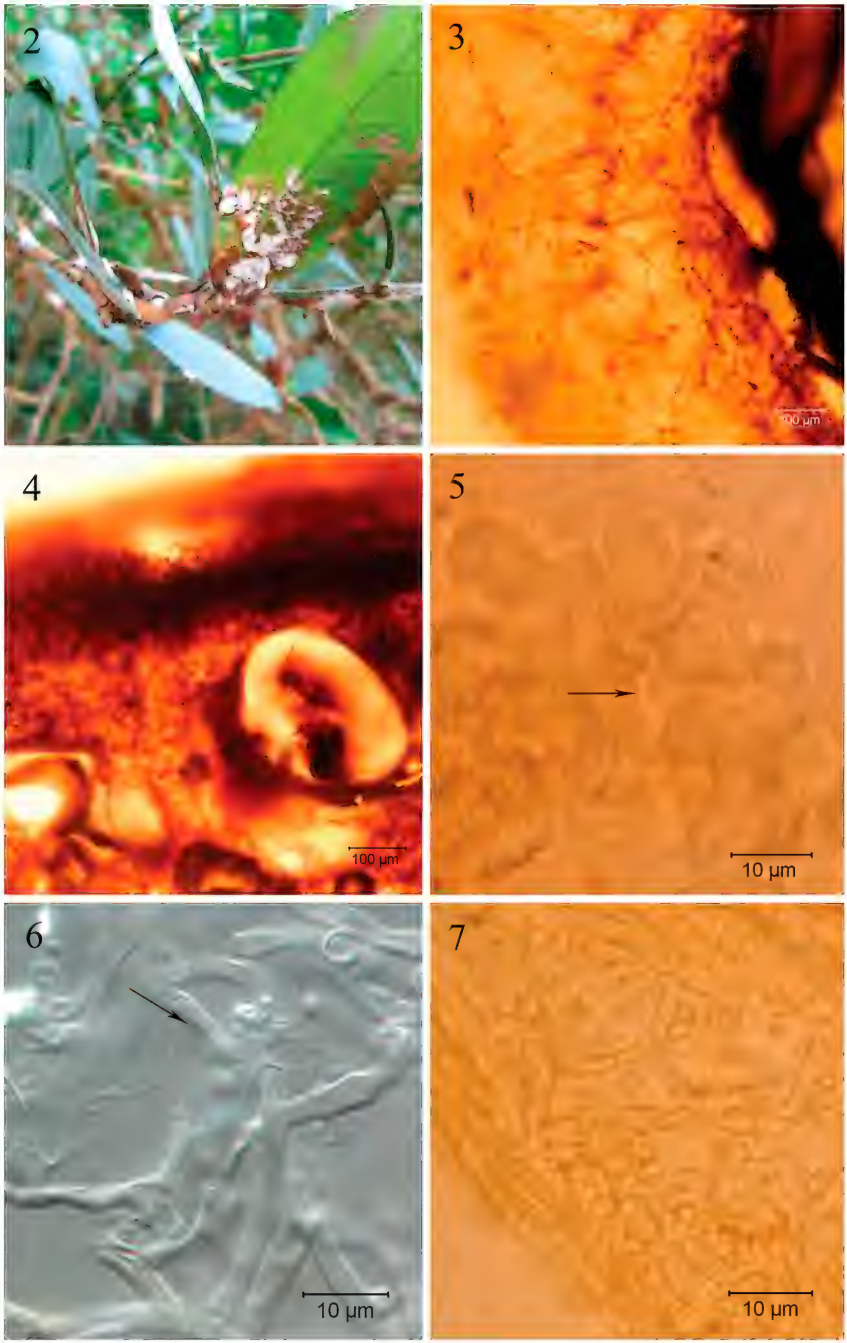


Fig. 1. Basidia of *Septobasidium atalantiae* (HMAS 251151, holotype).

cinnamon-brown; margin indeterminate, surface smooth or velutinous, frequently with round protuberance, sometimes cracked at maturity. In section 480–1200 μm thick. Subiculum brown, 25–100 μm thick. Pillars brown, 20–50 μm high, 20–70 μm wide. Towards the top the pillars branch out to form a hyphal layer, 420–830 μm high, sometimes with a bunch of hyphae in holes in the old stage. Hymenium hyaline, 60–70 μm thick. In the old stage from hymenial layer the fungal hyphae often renew growth to form the second hyphal layer and hymenium. Basidia arising directly from the hyphae without a probasidial cell, cylindrical, curved, 4-celled, 43–53 × 7.5–8.5 μm, hyaline. Sterigmata cylindrical or conical, 6–7 × 3–5 μm. Basidiospores not seen. Haustoria consisting of irregularly coiled hyphae, occasionally with closely packed parallel hyphae.

Figs. 2–7. *Septobasidium atalantiae* (HMAS 251151, holotype). 2. Basidiomata on branches and leaves. 3–4. Sections of basidiomata. 5–6. Basidia (arrows). 7. Haustoria.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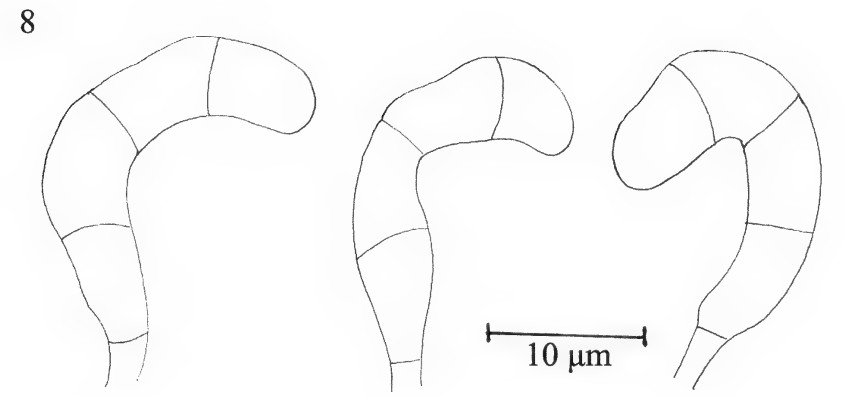


Fig. 8. Basidia of *Septobasidium henningsii* (HMAS 251152).

Remarks: Morphologically, *Septobasidium atalantiae* is similar to *S. natalense* Couch ex L.D. Gómez & Henk, but the latter has thinner section (250–500 mm high), taller pillars (100–200 μm high), and a non-stratose hymenial layer in the old stage (Couch 1938).

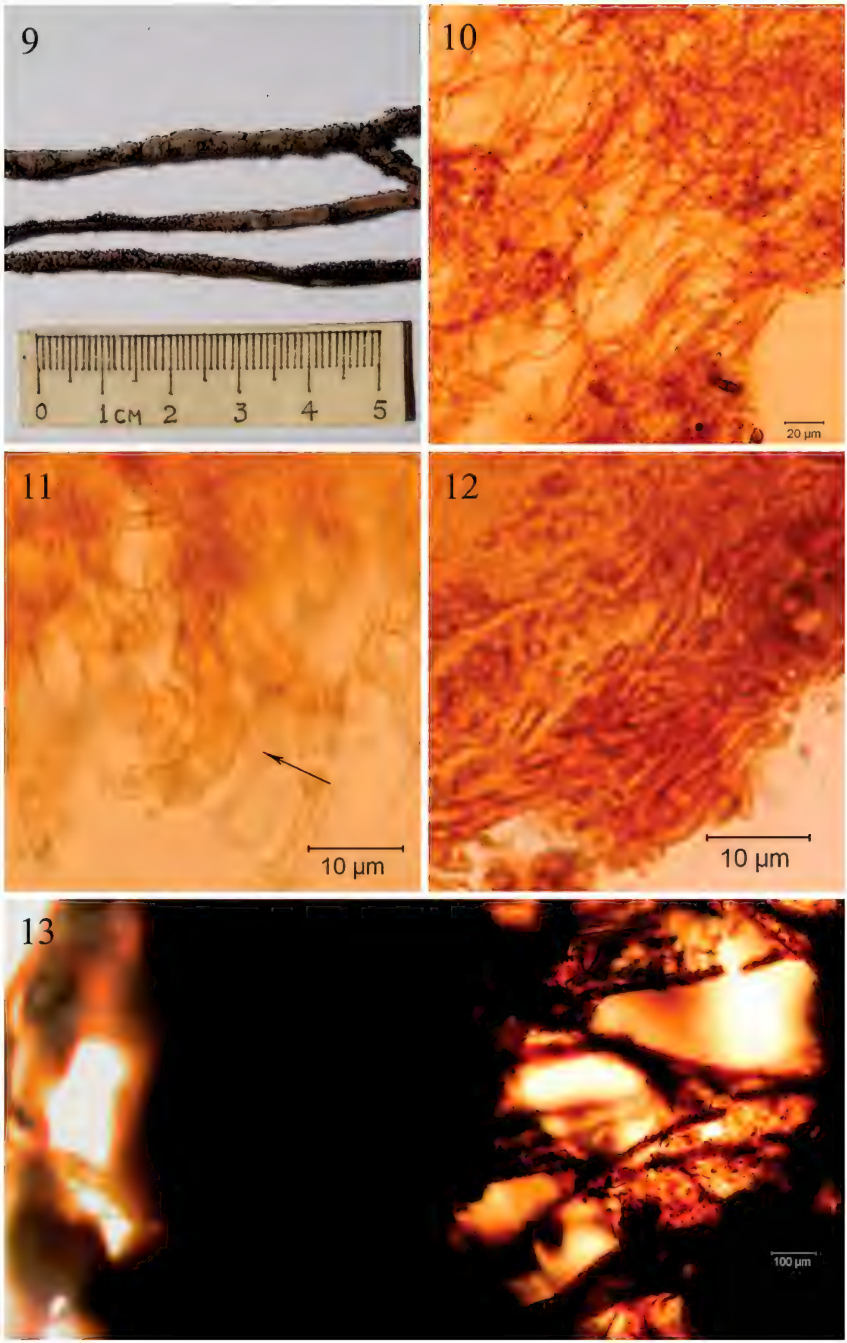
Septobasidium henningsii Pat., Monsunia 1: 138, 1899. Figs. 8–13

Basidiomata on branches, resupinate, (0.5–)5–12 cm long, 0.3–3 cm wide, brown or greyish brown; margin determinate, fibrillose, surface smooth, frequently with round protuberance, cracked at maturity. In section 1460–2200(–2500) μm thick. Subiculum brown, 25–50 μm thick. Pillars brown, 530–1100 μm high, 30–40 μm wide, entangled, arising in a slanting direction, often with a horizontal layer at the base of pillar. Hymenia brown, 650–850 μm thick, 1–4 strata, with closely packed parallel upright hyphae. Basidia at first pyriform or subglobose, cylindrical at maturity, straight or slightly curved, 4-celled, 23–38 × 5–6 μm, hyaline or yellowish, without a probasidial cell. Basidiospores not seen. Haustoria consisting of subfusiform cells or hyphae.

Specimen examined: On *Memecylon floribundum* Blume (*Melastomataceae*): **China**, Hainan Province, Bawangling Natural Reserve, alt. 370 m, 26.XI.2010, Y.F. Zhu & F. He 522, HMAS 251152.

Remarks: The Chinese specimen coincides morphologically with the original description of *Septobasidium henningsii* (Couch 1938), except that the original has larger (36–54 × 7–9.8 μm) basidia.

Figs. 9–13. *Septobasidium henningsii* (HMAS 251152). 9. Basidiomata on branches. 10. Hymenia. 11. Basidium (arrow). 12. Haustoria.13. Section of basidioma.


9
0 1CM 2 3 4 5
10
20 µm
11
10 µm
12
10 µm
13
100 µm

To date, 33 species of *Septobasidium* have been reported in China (Sawada 1933, Couch 1938, Teng 1963, Tai 1979, Kirschner & Chen 2007, Lu & Guo 2009a,b,c, 2010a,b,c, 2011, Lu et al. 2010, Chen & Guo 2011), including the two species reported in this paper.

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Revision of *Glomeromycetes* with entrophosporoid and glomoid spore formation with three new genera

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Abstract — New ribosomal gene analyses reveal that *Entrophospora* is non-monophyletic and its type species *E. infrequens* closely related to *Claroideoglomus* species, which supports transfer of the *Entrophosporaceae* from *Diversisporales* to *Glomerales* as well as the 'ancestral' *Claroideoglomus* spp. to *Albahypha* gen. nov. *Entrophospora baltica,* supported as a separate clade within *Diversisporales,* is designated as type species for the new monospecific *Sacculosporaceae. Entrophospora nevadensis,* phylogenetically close to *Diversipora* spp. and *Otospora bareae,* is transferred to *Tricispora* gen. nov. (*Diversiporaceae*). *Entrophospora*, *Sacculospora,* and *Tricispora* are morphologically distinguished by spore wall structure, pattern of the two spore pore closures proximal and distal to the sporiferous saccule, and relative spore and sporiferous saccule sizes. The shape of the white hyphae subtending the spore base separates *Albahypha* spp. from *Claroideoglomus* spp.

Key words — otosporoid, tricisporoid, evolution, molecular phylogeny, rDNA

Introduction

The genus *Entrophospora* was initially characterized according to intrahyphal spore formation within the neck of a sporiferous saccule (e.g. Ames & Schneider 1979, Błaszkowski et al. 1998, Palenzuela et al. 2010). Sieverding & Oehl (2006)

have transferred three species originally placed in *Entrophospora* (Schenck et al. 1984, Sieverding & Toro 1985, 1987, Wu et al. 1995) to new genera as *Intraspora schenckii* (Sieverd. & S. Toro) Oehl & Sieverd., *Kuklospora colombiana* (Spain & N.C. Schenck) Oehl & Sieverd., and *K. kentinensis* (C.G. Wu & Y.S. Liu) Oehl & Sieverd. Support for the re-classification included i) clear morphological differences from the type species, *Entrophospora infrequens* (Hall 1977; Ames & Schneider 1979; Sieverding & Oehl 2006); ii) morphological aspects placing the three species unequivocally in the *Acaulosporaceae* and *Archaeosporaceae*; and iii) neither spore morphology nor genetic analysis of *E. infrequens* support that species in *Acaulosporaceae*, where *Kuklospora* species phylogenetically belong (Sieverding & Oehl 2006).

Phylogenetic analyses showed that the recently described *E. nevadensis* belonged in *Diversisporaceae* (Palenzuela et al. 2010). To date *Diversisporaceae* includes 13 *Diversispora* and six *Redeckera* species that form diversisporoid spores on subtending hyphae (Oehl et al. 2011b) and one species with otosporoid spores formed laterally on the neck of sporiferous saccules (Palenzuela et al. 2008). Another newly described species, *Acaulospora colliculosa* Kaonongbua et al., was also believed to form its spores within the neck of sporiferous saccules (Kaonongbua et al. 2010) but the illustrations rather indicate a mixture of specimens of different species, isolated directly from field soil samples. Since no saccule was presented in the latter study, further observations are needed to confirm the existence of this species, which is assumed to form pacisporoid spores instead of kuklosporoid spores as indicated by the images.

Recent morphological and molecular analyses suggest that the genus *Entrophospora*, currently comprising three species, is not monophyletic, since *E. infrequens* and *E. nevadensis* phylogenetically belong to different orders, according to the recently revised *Glomeromycetes* (Oehl et al. 2011d). This means that of the original species in the genus, only *E. infrequens* and *E. baltica* (Błaszkowski et al. 1998) might remain in *Entrophospora*. Thus, one objective of the current study was to analyze and re-classify *Entrophospora* species based on combined morphological and phylogenetic analyses. In particular, we hoped to resolve the confusion regarding the sequence variabilities for *E. infrequens* noted by Millner et al. (2001) and Rodriguez et al. (2001). Another objective was to confirm the phylogenetic position of *Otospora bareae* (Palenzuela et al. 2008) within the *Diversisporaceae*.

Material & methods

Specimens analyzed by morphological means

Type and non-type material representing 30 arbuscular mycorrhizal (AM) fungal species currently placed in *Entrophosporaceae*, *Claroideoglomeraceae* and *Diversisporaceae* (Sieverding & Oehl 2006, Schüßler & Walker 2010, Oehl et al. 2011b) were analyzed (Table 1). Source material of *Entrophospora infrequens* was originated

from field samples and from trap and pure cultures obtained from several countries, with the pure cultures established in three different laboratories —EEZ, Granada, Spain; CIAT, Cali, Colombia; and Swiss Collection of Arbuscular mycorrhizal fungi (SAF) at Agroscope ART, Zurich, Switzerland (see Sieverding & Toro 1985, Palenzuela et al. 2010, Oehl et al. 2010a, 2011a). *Entrophospora baltica* was obtained from the type location in Poland and from several montane and alpine locations in Switzerland and Spain. *Entrophospora nevadensis* and *O. bareae* have so far been reported only from their type locations in the Sierra Nevada National Park and Sierra de Baza Natural Park in Andalucia (Spain), respectively (Palenzuela et al. 2008, 2010).

Table 1. Collections analysed for reorganizing *Entrophosporaceae, Claroideoglomeraceae,* and *Diversisporaceae.*

Basionym (species)	Type material (examiner)	Non-type material (examiner; coll. date or publication)
Entrophosporaceae		
Glomus infrequens	Type OSC (Oehl)	Specimen from USA, Brazil, Chile (Castillo et al. 2006), Colombia, Spain (Palenzuela; July 2004; pure culture established in 2006), Bolivia, Switzerland (Oehl et al. 2004, 2011a; August 2005)
Entrophospora baltica	Type (Oehl)	Specimen from Chile (Castillo et al. 2006), Spain (Palenzuela & Oehl; July 2007), Switzerland (Sieverding & Oehl 2006; July 2004)
E. nevadensis	Type (Oehl & Palenzuela; Palenzuela et al. 2010)	
Claroideoglomeraceae		
Glomus candidum	No access	
G. claroideum	Type OSC #40252 (Oehl)	Specimen from Brazil, Europe (Oehl et al. 2010b); cultures from Benin (Oehl; Tchabi et al. 2010)
G. drummondii	Type (Oehl), ex type (Goto)	
G. etunicatum	Holotype OSC (Oehl)	Specimen from Benin, Bolivia, Paraguay, Europe, Mexico (Oehl; Bashan et al. 2007)
G. lamellosum	Isotype OSC #50183 (Oehl)	Specimen from Poland, Germany (Oehl & Sieverding; Oehl et al. 2003, 2005)
G. luteum	Type OSC, ex type INVAM (Oehl)	
G. viscosum	Ex type (Oehl)	
G. walkeri	Type (Oehl), ex type (Goto)	
Diversisporaceae		
Diversispora celata	Ex type, inclusive pure cultures (Oehl; April 2009; deposited at SAF)	Specimen from Switzerland (Oehl; April 2009)
Glomus arenarium	Type at OSC (Oehl), ex type (Goto)	Specimen from Chile and UAE (Sieverding; Castillo et al. 2006)
G. aurantium	Type (Oehl)	Specimen from Germany (Oehl)
G. eburneum	Ex type at INVAM (Oehl)	Specimen from Bolivia & Oman (Oehl; Al-Yahya'ei et al. 2011)
G. epigaeum	Holotype OSC #39475 (Oehl)	
G. gibbosum	Type (Błaszkowski online pages)	Specimen from UAE (Sieverding, unpublished)

Table 1, concluded.

Basionym (species)	Type material (examiner)	Non-type material (examiner; coll. date or publication)
G. insculptum	Type (Oehl)	
G. przelewicense	No access	Specimen and pure pot cultures from Switzerland (Oehl & Sieverding; Oehl et al. 2009, published as *Glomus* sp. BR12)
G. pustulatum	Holotype OSC #46721 (Oehl)	Błaszkowski collection (Oehl)
G. spurcum	Ex type at INVAM (Oehl)	Specimen from Bolivia (Oehl; Nov. 2000)
G. avelingiae	No access	
G. canadense		Thaxter collection, Trappe collection (Oehl)
G. fragile		Trappe collection (Oehl)
G. fulvum		Trappe collection (Oehl), specimen from Brazil (Goto)
G. megalocarpum	Ex type (Oehl)	
G. pulvinatum		Trappe collection (Oehl)
G. tenerum		Specimen from Australia (Oehl; McGee & Trappe 2002)
G. trimurales	Holotype OSC #49584 (Oehl)	
Endogone versiformis		Specimen at INVAM; specimen from Central Europe (Oehl & Sieverding; Oehl et al. 2003)
Otospora bareae	Type (Oehl & Palenzuela; Palenzuela et al. 2008)	

Morphological analyses

Morphological observations of spores (including sporiferous saccules and subcellular structures), were based on freshly prepared specimens mounted in polyvinyl alcohol-lactic acid-glycerol (PVLG), in a mixture of PVLG and Melzer's reagent (Brundrett et al. 1994), in a 1:1 (v/v) mixture of lactic acid and water, in Melzer's reagent, or in water. Spore structure terminology for species with acaulosporoid, entrophosporoid, or glomoid spore formation follows Goto & Maia (2006), Sieverding & Oehl (2006), Oehl et al. (2006, 2011b), Spain et al. (2006), and Palenzuela et al. (2008, 2011). Photographs were taken using an Olympus digital camera (model DP70-CU) mounted on a Zeiss Axioplan compound microscope.

Molecular and phylogenetic analyses

Materials selected for molecular analyses included: *E. infrequens*— spores from trap and pure culture material maintained at EEZ (Granada, Spain) and SAF (Zurich, Switzerland); *E. baltica*— trap cultures and field samples from Mulhacen in Sierra Nevada (Southern Spain, 3200 m asl) and Piz Corvo (Passo del Lugmagno, Swiss Central Alps, 2700 m asl); *E. nevadensis*— EEZ pure culture (Palenzuela et al. 2010); *O. bareae*— EEZ maintained trap cultures (Palenzuela et al. 2008).

DNA extraction, amplification and sequencing from Spanish isolates were performed at EEZ (Palenzuela et al. 2011) and from non-Spanish isolates at UFPE (Recife; see Goto et al. 2011, Oehl et al. 2011c).

For sequence alignment we first verified the National Center for Biotechnology Information (NCBI) databases using the BLASTn program to ensure that the *E. infrequens* sequences were affiliated to *Claroideoglomeraceae* (*Glomerales*), and that the sequences

from *E. baltica, E. nevadensis* and *O. bareae* correctly aligned with *Diversisporales*. AM fungal rRNA sequences (SSU = ~1770 bp; LSU = 733 bp) obtained in our laboratories were then aligned with GenBank glomeromycotean sequences using ClustalX (Larkin et al. 2007) and edited with BioEdit (Hall 1999) to obtain a final alignment. The sequences were deposited at GenBank under the accession numbers FR865452–FR865456 and JN113035-JN113037 for *E. infrequens*, FR865457–FR865462 and FR865449–FR865451 for *E. baltica*, FR865465–FR865467 and FR865446–FR865448 for *E. nevadensis,* and FR8655463–FR865464 and FR865444–FR865445 for *O. barea.*

Maximum parsimony (MP) and neighbor joining (NJ) analyses with 1000 bootstrap replications were performed using the Phylogenetic Analysis Using Parsimony (PAUP) vers. 4 (Swofford 2003). Bayesian (two runs over 1 x 10^6 generations with a burnin value of 2500) and maximum likelihood (1000 bootstrap) analyses were performed, respectively, in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003), launched from Topali 2.5. The nucleotide substitution model was estimated using Topali 2.5 (Milne et al. 2004). Sequences from *Neurospora crassa* Shear & B.O. Dodge and *Boletus edulis* Bull. were used as outgroups for *Glomeromycota.*

Results

Molecular phylogenetic analyses

The phylogenetic analyses on the nearly complete SSU of the ribosomal gene and on partial sequences of the LSU show that *E. infrequens*, type species of the *Entrophosporaceae* (Sieverding & Oehl 2006), is related to *Claroideoglomus* species in the *Glomerales* (Figs 1–2). However, *E. infrequens* is phylogenetically unrelated to the two other *Entrophospora* species that form two monophyletic clades in the *Diversisporales* (Figs 1–2). *Entrophospora nevadensis* groups next to *O. bareae* and several *Diversispora* species, while *E. baltica* groups in its own clade, well distant from the other major clades representing families within the *Diversisporales*. The phylogenetic analyses also render the genus *Diversispora* paraphyletic (Figs 1–2).

Morphological comparisons

For the *E. infrequens*, *E. baltica*, *E. nevadensis*, and *Otospora bareae* clades and the two *Claroideoglomus* clades, some morphological characters are congruent with the phylogenetic findings. These characters are principally the sporiferous saccule characteristics, pore closure patterns at the spore bases for species forming spores within/on the saccule necks (Figs 3–19), and spore base and subtending hyphae characteristics for species forming spores terminally on hyphae (Figs 20–31).

Entrophospora infrequens forms saccules that are regularly larger than the spores formed beneath, and the pore formed by the subtending hypha distal to the saccules is closed by hyaline evanescent outer spore wall layers; the next-inner structural pigmented persistent layer does not continue into the distal part of the hyphal neck and never forms a pore distally to the saccule

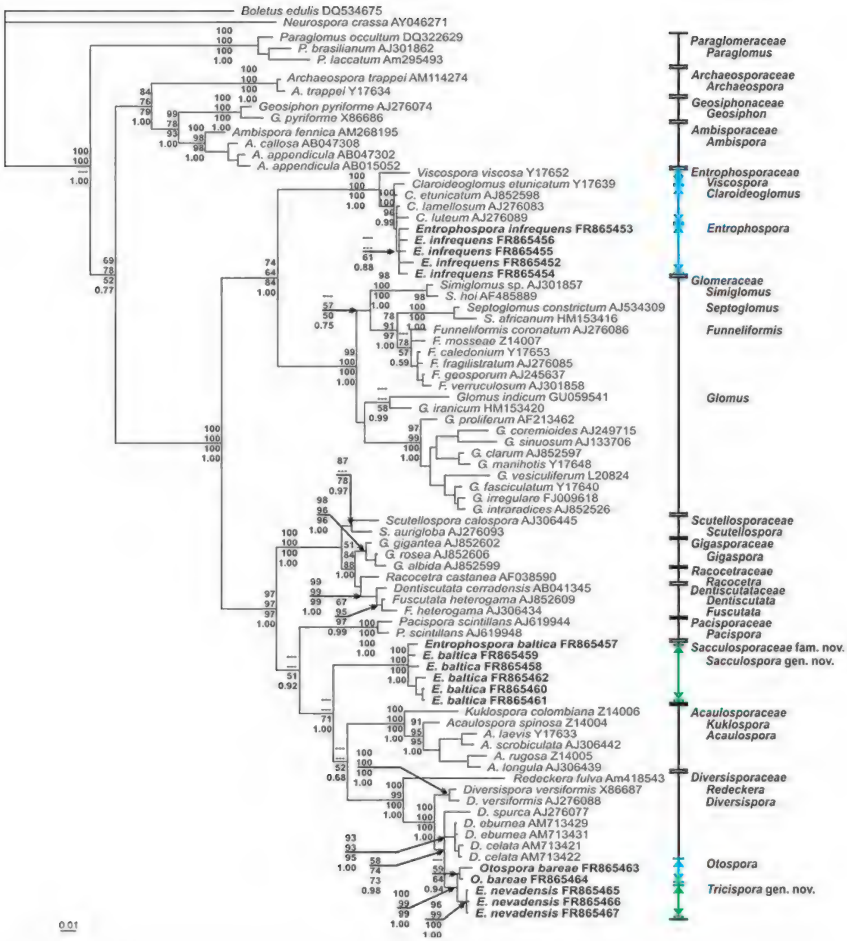


FIG. 1. A phylogeny of the *Glomeromycota* based on partial SSU rDNA sequences (~1800 bp). NJ (neighbor joining), ML (maximum likelihood), and Bayesian analyses were performed with GTR+G+I substitution model. Sequence labels correspond to their database accession numbers. Support values are from NJ, MP (maximum parsimony), ML, and Bayesian analyses. New sequences obtained in this study are indicated in bold. Only topologies with bootstrap values ≥ 50% are shown. Consistency Index = 0.47; Retention Index = 0.84.

(FIGS 3–7). Thus, on the spores (particularly once the outer wall layers have become degraded) just one single opening/pore forming a 'cicatrix' is visible proximal to the saccules; the pore is closed by a plug-like wall material derived apparently from the structural wall layer (OWL3; FIGS 3–4). The structural wall layer extends into the saccule over 10–30 µm, with the spore wall layer

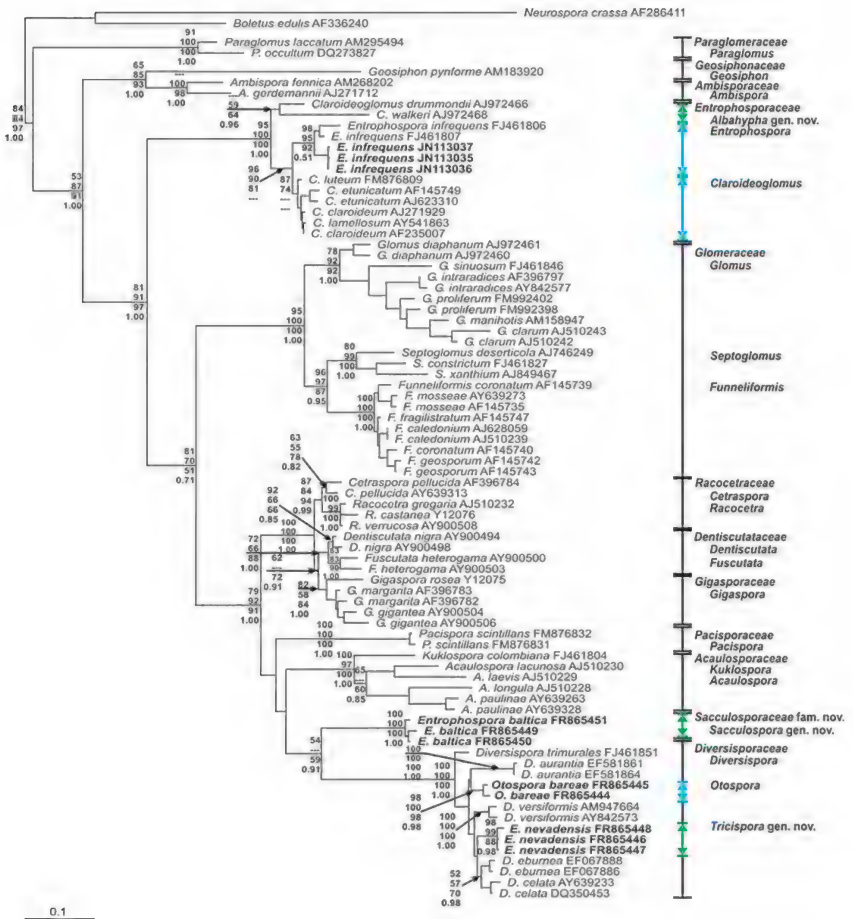


FIG. 2. A phylogeny of the *Glomeromycota* based on partial LSU rDNA sequences (~600 bp). NJ (neighbor joining), ML (maximum likelihood), and Bayesian analyses were performed with GTR + G substitution model. Sequence labels correspond to their database accession numbers. Support values are from NJ, MP (maximum parsimony), ML, and Bayesian analyses. New sequences obtained in this study are indicated in bold. Only topologies with bootstrap values ≥ 50% are shown. Consistency Index = 0.41; Retention Index = 0.79.

ornamentation apparently continuing to the pore closure area, although not within the saccule.

Entrophospora baltica and *E. nevadensis* (FIGS 8–19) have smooth proximal cicatrices; here the ornamentation does not continue at the area of the generally wide pore proximal to the sporiferous saccule. Both the distal and proximal

spore pores are closed by a smooth, rather thin septum of the structural, laminate wall layer, and the structural wall layer continues for a small distance into the distal hypha of the saccule neck (Figs 8, 12, 16). In both species the saccules are often slightly (*E. baltica*) or substantially (*E. nevadensis*) smaller than spores formed beneath since they sometimes equal half or a quarter of the spore diameter, respectively. Remarkably, detailed spore wall structure analyses revealed that *E. baltica* has three walls (Figs 17–19), while spores of *E. nevadensis* (and *Otospora bareae*) are clearly bi-layered (Fig. 13, and Palenzuela et al. 2010).

Typical *Claroideoglomus* species (e.g., *C. etunicatum*, *C. claroideum*, *C. lamellosum* (Figs 20–25), *C. luteum*) form hyaline subtending hyphae, which are 'funnel-shaped' — their width at the spore base is regularly > 2.5 times wider than 10–50 µm distant from the spore. However, two species that are basal within the *Claroideoglomeraceae* — *C. drummondii*, *C. walkeri* (Fig. 2, Oehl el al. 2011b)— have only slightly funnel-shaped to sometimes cylindrical subtending hyphae that are regularly < 2.0 times broader at spore base than the supporting hypha (Figs 26–31).

Based on our phylogenetic analyses, we propose to include the *Entrophosporaceae* and its type species *E. infrequens* in the *Glomerales*. The LSU rRNA sequence analyses indicate that *Claroideoglomus* should be further divided into two genera, which is supported by the morphological differences between the two recognized clades.

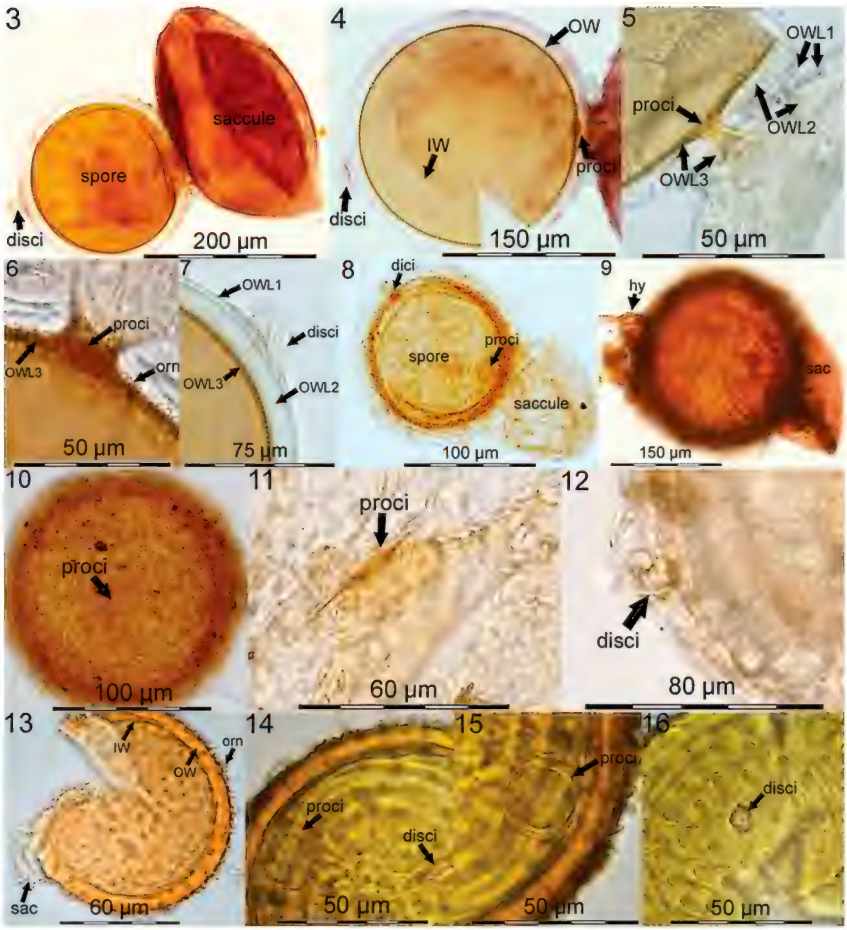
Since *E. nevadensis* forms a clade within the *Diversisporaceae,* a new genus is indicated with it serving as type species. *Entrophospora baltica*, which forms a monophyletic major clade within the *Diversisporales,* should serve as the type species of a new family in the order.

Sequence analyses further imply that *Diversispora* is paraphyletic. However, here the molecular and morphological databases do not yet provide sufficient information to reclassify accurately the *Diversispora* species.

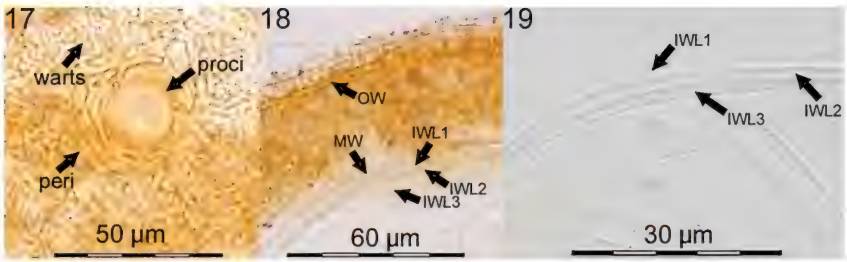
Taxonomic revision

Glomerales J.B. Morton & Benny, emend. Oehl, Palenz., G.A. Silva & Sieverd.

Emended description: Spores form terminally on or intercalary in hyphae or within the necks of sporiferous saccules in soil (or sometimes roots) singly or (when glomoid) also in spore clusters or multi-spored loose to compact sporocarps; when in compact sporocarps (with or without peridium), spores randomly distributed or organized around a central hyphal plexus. Glomoid spores with one single or multiple-layered wall. Entrophosporoid spores with two walls: outer structural wall and inner (germinal) wall. In glomoid spores, wall of the subtending hyphae (sh) conspicuously continuous with the spore wall, sh funnel-shaped, cylindrical, or constricted and concolorous with



Figs 3–16. Examples of intra-hyphal spore formation within the neck of a sporiferous saccule. Figs 3–7. *Entrophospora infrequens*: Spores have two walls (ow & iw); saccule generally larger than attached spore. ow is triple-layered (owl1–3) and has a characteristic ornamentation ('orn') on structural layer owl3. At spore bases two cicatrices are formed: proximal and distal to the saccule. The persistent proximal cicatrix ('proci') formed by the persistent owl3 (Figs 5–6) wall material resembles a plug. The evanescent distal cicatrix ('disci') is formed by evanescent owl2 (Fig. 7) material. Figs 8–12. *Sacculospora baltica*: spores with two cicatrices; saccule (sac) substantially smaller than attached spore. The proximal cicatrix (proci) is persistent and formed by the structural ow layer (Figs 10–11); the corresponding spore pore is closed by a septum that does not resemble a plug. The distal cicatrix ('dici') is also persistent and formed by the same layer (Fig. 12). Figs 13–16. *Tricispora nevadensis*: spores have two walls (ow & iw) and two cicatrices; saccule (sac) substantially smaller than the attached spore. The proximal cicatrix (proci) is persistent and formed by the structural ow layer (Figs 14–15); the corresponding spore pore is closed by a septum that does not resemble a plug. The distal cicatrix (dici) is also persistent and formed by the same layer (Fig. 16).



Figs 17–19. *Sacculospora baltica* spore wall structure showing peridial hyphal mantle (peri), conspicuous proximal cicatrix (proxy), and warts on the outer wall (OW) surface; middle wall (MW) is 1(–2)-layered and inner wall is triple-layered (IWL1–3). The three IW layers generally tightly adhere, making IWL1 and IWL3 difficult to observe (Fig. 18); the separation of the three layers in Fig. 19 is an artifact of cover-slip pressure.

spore, slightly paler, or (sub-)hyaline. In entrophosporoid spores, structural pigmented outer wall layer discontinuous with the hyphal wall distal to the saccule; forming typical vesicular-arbuscular mycorrhiza with mycorrhizal structures that stain blue to dark blue in trypan blue.

Type family: *Glomeraceae* Piroz. & Dalpé

Other family: *Entrophosporaceae* Oehl & Sieverd.

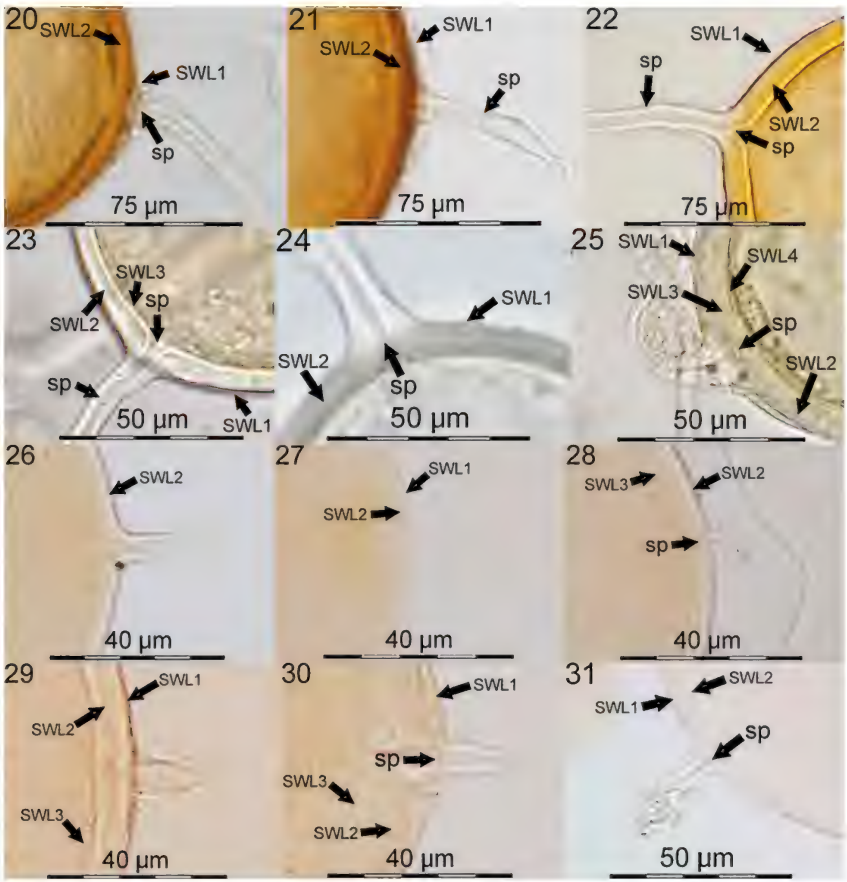
Entrophosporaceae Oehl & Sieverd., emend. Oehl, Sieverd., Palenz. & G.A. Silva

= *Claroideoglomeraceae* C. Walker & A. Schüssler, The *Glomeromycota* – a species list: 21. 2010.

Emended description: Spore formation is of glomoid or entrophosporoid type. Glomoid spores form in soil or (rarely) in roots singly, in clusters with few spores, or (extremely rarely) in sporocarps; SH hyaline to white, rarely subhyaline, often conspicuously funnel- or bill-shaped. Spores with one wall of 1–4 layers; spore base pore closure often with a septum that arises from the structural layer, an adherent thin inner layer, or both layers. Entrophosporoid spores form singly in soils or rarely in roots, subterminally or intercalary within the neck of a tightly attached sporiferous saccule that generally is larger in size than the underlying spore; they have two walls: outer and inner. Outer, hyaline, semi-persistent to evanescent layers of the outer spore wall are the hyphal neck and sporiferous saccule wall layers. The pigmented structural layer does not continue within the hyphal wall but only within the saccule terminus for some distance. Pore closed by a plug towards the saccule. The inner wall is generally thick, finely laminated and forms de novo. No inner wall layer has a beaded appearance or stains in Melzer's reagent. Fungal structures in roots stain blue with trypan blue; forming vesicular-arbuscular mycorrhizae.

Type genus: *Entrophospora* R.N. Ames & R.W. Schneid.

Other genera: *Claroideoglomus* C. Walker & A. Schüssler, *Albahypha* Oehl et al., *Viscospora* Sieverd. et al.



Figs 20–31. Examples of terminal spore formation on subtending, hyaline to white hyphae (SH); spores with 2–4 layers (SWL1–4); spore pores regularly closed by a septum (sp) at the spore base. Figs 20–25. *Claroideoglomus* (Figs 20–22: *C. etunicatum,* Figs 23–24: *C. claroideum,* Fig. 25: *C. lamellosum*) — *Claroideoglomus* spores have a significantly funnel-shaped hypha with structural wall layer (generally SWL2 or SWL3) that is >2.5 times thicker at spore base than 10–25 µm distant at transition between SH and mycelia hypha. Figs 26–31. *Albahypha* (Figs 26–28: *A. drummondii,* Figs 29–31: *A. walkeri*) — *Albahypha* spores have a slightly funnel-shaped to cylindrical hypha with structural wall layer (generally SWL2) that is <2.0 times thicker at spore base than 10–25 µm distant.

Entrophospora R.N. Ames & R.W. Schneid., emend. Oehl, Sieverd., Palenz. & G.A. Silva Figs 1–5

Emended description: Sporocarps unknown. Entrophosporoid spores form within the hyphal neck of tightly attached terminal or intercalary sporiferous saccules, singly in soils, or (rarely) in roots. Sporiferous saccules generally are

larger in size than the underlying spores. Entrophosporoid spores are globose to subglobose and have two walls: an outer and an inner. Outer, semi-persistent to evanescent layers of the outer spore wall are the wall layers of the hyphal stalk and the sporiferous saccule. The structural, pigmented layer beneath does not continue within the hyphal wall but only for a short distance within the saccule terminus. Thus, spores have only one persistent cicatrix, which is proximal to the globose saccule terminus. A plug closes the pore towards the saccule. The inner wall is thick, finely laminated wall and forms de novo. No inner wall layers have a beaded appearance. Fungal structures in roots stain blue with trypan blue; forming vesicular-arbuscular mycorrhizae.

Type species: *Entrophospora infrequens* (I.R. Hall) R.N. Ames & R.W. Schneid.

Claroideoglomus C. Walker & A. Schüssler, emend. Oehl, Sieverd., B.T. Goto & G.A. Silva Figs 20–25

Emended description: Spores formed on subtending hyphae (sh), generally singly in soil or rarely in roots; sh are hyaline to white, rarely subhyaline, and funnel- or bill-shaped with widths > 2.5 times greater at the spore base than at 10–20 µm from the spore. Spores with one wall of 1–4 layers; pore closure at spore base often with a septum that arises species-specifically from the structural layer, an adherent thin innermost layer, or both innermost layers.

Type species: *Claroideoglomus claroideum* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüssler

Albahypha Oehl, G.A. Silva, B.T. Goto & Sieverd., **gen. nov.** Figs 26–31

MycoBank MB 561639

Sporae singulariter efformatae; tunica sporarum cum tunica hypharum coniuncta; hyphae albae, tunica hyphae 1.2–2.0 maior ad basem sporarum quam in 10-20 µm distantia basae sporarum; porum sporarum occlusum septa tunicae sporarum, rarum apertum. Mycorrhizas vesiculares-arbusculares formans caeruleas colorantes cum 'trypan blue'.

Type species: *Albahypha drummondii* (Błaszk. & Renker) Sieverd. et al.

Etymology: derived from the Latin: *alba* = white; *hypha* = hypha; referring to the white, slightly funnel-shaped subtending hypha which is characteristic for species of this genus.

Key characters: Spores formed generally singly in soil or rarely in roots; sh white, rarely subhyaline, 1.2–2.0 times wider at spore base than their width 10–20 µm distance from the spore, giving a slightly funnel-shaped or cylindrical appearance. Spores with one wall of 1–4 layers; spore base pore closure often with a septum that may arise from the structural layer, an adherent innermost, (semi-)flexible layer, or both innermost layers.

Albahypha drummondii (Błaszk. & Renker) Sieverd., Oehl, B.T. Goto & G.A. Silva, **comb. nov.**

MycoBank MB 561640

≡ *Glomus drummondii* Błaszk. & Renker, Mycol. Res. 110: 559. 2006.

≡ *Claroideoglomus drummondii* (Błaszk. & Renker) C. Walker & A. Schüssler, The *Glomeromycota* – a species list: 22. 2010.

Albahypha walkeri (Błaszk. & Renker) Sieverd., Oehl, B.T. Goto & G.A. Silva, **comb. nov.**

MycoBank MB 561641

≡ *Glomus walkeri* Błaszk. & Renker. Mycol. Res. 110: 563. 2006.

≡ *Claroideoglomus walkeri* (Błaszk. & Renker) C. Walker & A. Schüssler, The *Glomeromycota* – a species list: 22. 2010.

Viscospora Sieverd., Oehl & G.A. Silva, Mycotaxon 116: 108. 2011.

Type species: *Viscospora viscosa* (T.H. Nicolson) Sieverd. et al.

Diversisporaceae C. Walker & A. Schüssler, emend. Oehl, Palenz., I.C. Sánchez, G.A. Silva, B.T. Goto & Sieverd.

Emended description: Spore formation diversisporoid, otosporoid, or entrophosporoid sensu lato ('tricisporoid' sensu stricto). Diversisporoid spores formed singly, in clusters, or in large disorganized sporocarps with high spore numbers. In pigmented spores, subtending hyphae (sh) conspicuously change color, becoming hyaline to white behind the septum, (immediately or at a very short distance from this septum); sh generally straight, cylindrical, in some species constricted or inflated. Spores with 1–3 wall layers; pore often closed with a septum that may arise from innermost wall lamina, an overlaying laminate layer, or from both; sh pore rarely open. Otosporoid and tricisporoid spores with two multiple-layered walls; otosporoid spores formed laterally on the persistent neck of a terminal or intercalary sporiferous saccule at some distance from the saccule terminus; spore pore generally closed by a septum at spore base. Tricisporoid spores formed within the evanescent neck of a tightly attached terminal or intercalary sporiferous saccule, closely attached to the saccule terminus which is often smaller in size than the mature spores attached, rarely equal in size; tricisporoid spores with two cicatrices formed by the outer wall pigmented structural layer.

Type genus: *Diversispora* C. Walker & A. Schüssler

Other genera: *Redeckera* C. Walker & A. Schüssler, *Otospora* Oehl et al., *Tricispora* Oehl et al.

Diversispora C. Walker & A. Schüssler, Mycol. Res. 108: 982. 2004.

Type species: *Diversispora spurca* (C.M. Peiff. et al.) C. Walker & A. Schüssler

Otospora Oehl, Palenz. & N. Ferrol, Mycologia 100: 297. 2008.

Type species: *Otospora bareae* Palenz. et al.

Redeckera C. Walker & A. Schüssler, The *Glomeromycota* – a species list: 44. 2010.

Type species: *Redeckera fulva* (Berk. & Broome) C. Walker & A. Schüssler

Tricispora Oehl, Sieverd., G.A. Silva & Palenz., **gen. nov.** FIGS 13–16

MYCOBANK MB 561642

Sporocarpia ignota. Sporae singulatim efformatae subterminaliter vel intercalariter in hypha inflata anguste adiacetum ad sacculum sporiferum terminalem vel intercalarem. Sacculus sporiferus frequenter minor quam sporae globosae vel subglobosae; sporae duabus tunicis stratis pluribus. Stratum exterior tunicae exterioris coniunctum tunica hyphae et sacculi. Stratum interiorem tunicae exterioris laminatum, duas poras sporae occludens. Stratum exterior tunicae interioris non granulatum. Formans mycorrhizas vesicular-arbusculares. Structurae fungorum colorantes caeruleae cum 'trypan blue'.

TYPE SPECIES: *Tricispora nevadensis* (Palenz. et al.) Oehl et al.

ETYMOLOGY: derived from the Latin: *(cica-)trix* = cicatrix: and *spora* = spore; referring to the two conspicuous cicatrices left on the structural wall layer of the spores, even when the sporiferous saccules and the hyphal neck distal to the saccule have detached completely from the spores.

KEY CHARACTERS: Sporocarps unknown. Spores formed within the hyphal neck of closely adherent terminal or intercalary sporiferous saccules. The globose saccule terminus generally is substantially smaller than the attached mature spore. Spores have an outer and an inner wall. At least two layers (including the outer wall structural layer) are continuous with the sporiferous saccule wall. The outer layer of the outer wall is evanescent, the inner layers are permanent. After the hyphal neck connections break off, spores show two, often opposite, cicatrices that are closed by the permanent sublayers of the outer wall structural layer. The inner wall forms de novo, consists of several layers without granular ('beaded') appearance and does not stain with Melzer's reagent. The fungal structures in the roots stain blue to dark blue with trypan blue; forming vesicular-arbuscular mycorrhiza.

Tricispora nevadensis (Palenz., N. Ferrol, Azcón-Aguilar & Oehl) Oehl, Palenz., G.A. Silva & Sieverd., **comb. nov.**

MYCOBANK MB 561644

≡ *Entrophospora nevadensis* Palenz., N. Ferrol, Azcón-Aguilar & Oehl, Mycologia 102(3): 627. 2010.

Sacculosporaceae Oehl, Sieverd., G.A. Silva, B.T. Goto, I.C. Sánchez & Palenz., **fam. nov.**

MYCOBANK MB 561645

Sporae singulatim efformatae in hypha inflata anguste adiacetum ad sacculum sporiferum terminalem vel intercalarem. Sporae tribus tunicis stratis pluribus. Stratum interiorem tunicae exterioris laminatum, duas poras sporae occludens. Stratum exterior tunicae interioris non granulatum.

KEY CHARACTERS: Sporocarps unknown. Spores formed within the hyphal neck of closely adherent terminal or intercalary sporiferous saccules. Spores have three walls: outer, middle and inner. At least two layers (including the outer wall structural layer) are continuous with the sporiferous saccule wall. After the

hyphal neck connections break off, spores show two, often opposite, cicatrices that are closed by the permanent sublayers of the outer wall structural layer. Middle and inner wall form de novo. Middle wall is 1–2-layered. Inner wall consists of several layers, none of which has a granular ('beaded') appearance, and does not stain in Melzer's reagent.

Type genus: *Sacculospora* Oehl et al.

Sacculospora Oehl, Sieverd., G.A. Silva, B.T. Goto, I.C. Sánchez & Palenz., **gen. nov.**

MycoBank MB 561646 Figs 8–12, 17–19

Sporocarpia ignota. Sporae singulatim efformatae subterminaliter vel intercalariter in hypha inflata anguste adiacetum ad sacculum sporiferum terminalem vel intercalarem. Sporae globosae vel subglobosae, tribus tunicis stratis pluribus. Stratum exterior tunicae exterioris coniunctum tunica hyphae et sacculi. Stratum interiorem tunicae exterioris laminatum, duas poras sporae occludens. Stratum exterior tunicae interioris non granulatum.

Type species: *Sacculospora baltica* (Błaszk. et al.) Oehl et al.

Etymology: derived from the Latin: *sacculus* = saccule: and *spora* = spore; referring to the spore formation within the neck of sporiferous saccules.

Key characters: Sporocarps unknown. Spores formed within the hyphal neck of closely adherent, terminal or intercalary sporiferous saccules. Spores have three walls: outer, middle and inner. At least two layers (including the outer wall structural layer) are continuous with the sporiferous saccule wall. Inner layers of the outer spore wall are permanent. After the hyphal neck connections break off, spores show two, often opposite, cicatrices that are closed by the permanent sublayers of the outer wall structural layer. Middle and inner wall form de novo. Middle wall is 1–2-layered. Inner wall consists of several layers, none of which have a granular ('beaded') appearance (Fig. 18–19), and does not stain in Melzer's reagent. The inner wall may be germinal in function, but a germination structure has not yet been found.

Sacculospora baltica (Błaszk., Madej & Tadych) Oehl, Palenz., I.C. Sánchez, B.T. Goto, G.A. Silva & Sieverd., **comb. nov.**

MycoBank MB 561647

≡ *Entrophospora baltica* Błaszk., Madej & Tadych, Mycotaxon 68: 167. 1998.

Discussion

New molecular and morphological analyses elucidated the molecular phylogenetic and morphological congruencies within a group of species that have long been considered heterogeneous (e.g. Morton & Benny 1990, Sieverding & Oehl 2006). Species with entrophosporoid (sensu lato) spore formation are now found within *Entrophosporaceae* (*E. infrequens*), *Acaulosporaceae* (*K. colombiana, K. kentinensis*), *Diversisporaceae* (*T. nevadensis*), *Sacculosporaceae* (*S. baltica*), and *Archaeosporaceae* (*Intraspora schenckii*).

Sequence analyses of *E. infrequens*, with a unique entrophosporoid spore formation, place this species so close to *Claroideoglomus* that we regard

the recently described *Claroideoglomeraceae* as a heterotypic synonym of *Entrophosporaceae*. Morphological congruencies were found to support the phylogeny and helped identify a fourth genus, *Albahypha*, to include with the type genus *Entrophospora*, *Claroideoglomus,* and *Viscospora* within the revised *Entrophosporaceae.*

The *Diversisporaceae* now comprise four genera: two with diversisporoid spore formation (*Diversispora* and *Redeckera*; Oehl et al. 2011b) and one each with otosporoid (*Otospora*) and tricisporoid (*Tricispora*) spore formation.

There are now several phylogenetic clades that form spores terminally on hyphae as well as on or within sporiferous saccules (spore formation type glomoid s.l., acaulosporoid s.l., entrophosporoid s.l.). These are represented in the *Entrophosporaceae* (*Glomerales*), *Diversisporaceae* (*Diversisporales*), and *Archaeosporaceae* and *Ambisporaceae* (*Archaeosporales*) clades but only *Diversisporaceae*, *Archaeosporaceae* and *Ambisporaceae* are represented by all three major spore formation types.

Further studies are still needed to determine whether the entrophosporoid (sensu lato) genus *Kuklospora* is monophyletic. Most phylogenetic analyses split the type species, *K. colombiana*, off at the base of *Acaulosporaceae* (Schüßler et al. 2001, Palenzuela et al. 2008, 2010, Oehl et al. 2011d). Thus, we conclude that the synonymization of *Kuklospora* with *Acaulospora* (Kaonongbua et al. 2010) is not justified and needs to be substantiated (Oehl et al. 2011e). Moreover, a revision of the phylogenetically and morphologically heterogeneous *Acaulosporaceae* is urgently needed (Spain 1992, Oehl et al. 2011e).

Our analyses supports *Tricispora nevadensis* (≡ *E. nevadensis*) and *Otospora bareae* as sister taxa within the *Diversisporaceae* (Figs 1–2). Both *Entrophospora* and *Otospora* can readily be distinguished by spore formation type, distance between the saccule terminus and the differentiated spore within (*Entrophospora*) or laterally (*Otospora*) on the saccule neck, and the relative size differences between saccule and spore. Morphologically, these genera have little in common with the type genus of their family, *Diversispora*, whose species form spores simply on subtending hyphae. We emphasize that there have never been genera in *Glomeromycetes* (sensu Oehl et al. 2011d) that encompass species with both glomoid and entrophosporoid or acaulosporoid spore formation. This strongly supports the proposition of *Otospora* and *Tricispora*. However, phylogenetically, both *T. nevadensis* and *O. bareae* render the genus *Diversispora* polyphyletic.

We regard the *Diversispora* molecular database as still rudimentary, as the genus comprises several species that yet to be molecularly analyzed (Oehl et al. 2011b). Additionally, the morphological differences among *Diversispora* clades (Figs 1–2) are not yet clear. Before the genus *Diversispora* can confidently be reclassified, additional molecular and morphological studies are needed to

determine relationships accurately within this heterogeneous family.

Glomus hyderabadense Swarupa et al. also deserves mention, for it regularly forms a small terminal spore and an adjacent, subterminal large spore on subtending hyphae. Since the terminal spore is generally much smaller than the subterminal spore (Swarupa et al. 2004), its spore formation superficially resembles that of *T. nevadensis*. Although the *G. hyderabadense* sequence (AY211274) aligns adjacent to *G. clarum* (Swarupa et al. 2004), the deposited sequence does not represent a glomeromycotan fungus. *Glomus hyderabadense* possibly forms its own clade within the *Glomeromycetes* and thus may represent an undescribed fungal genus with a unique, diagnostic spore formation and morphology.

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Puccinia cortusae (*Basidiomycota*; *Uredinales*) on *Cortusa brotheri* (*Primulaceae*), new to southern Asia (Fairy Meadows, Pakistan)

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Abstract — *Puccinia cortusae* has been found parasitizing *Cortusa brotheri* from northern Pakistan. This first report from Pakistan and southern Asia also establishes *C. brotheri* as a new host for the rust fungus. We believe this is the first report of *P. cortusae* since it was first described in 1904. New observations of one-celled teliospores and variable germ pore position supplement the original description.

Key words — Bial Camp, *Cortusa matthioli*

Introduction

Worldwide, the *Primulaceae* comprises ca. 24 genera and 1000 species, (most of which represent herbs confined mainly to north temperate regions), among which only around 28 species have been recorded as alternate or (sometimes) primary hosts for 18 rust species and five genera (Arthur & Cummins 1962, Wilson and Henderson 1966, Hiratsuka et al. 1992, Farr & Rossman 2011). Although 60 species (eight genera) representatives of the *Primulaceae* have been reported from Pakistan, not a single species has been from the country as a host for rust fungi.

During the present study, *Cortusa brotheri* was found parasitized by *Puccinia cortusae*. With this addition, the number of rust fungi has been raised to 73 from Fairy Meadows, Pakistan (Afshan et al. 2009, 2011).

Materials & methods

During the survey of rust fungi from Pakistan, *Cortusa brotheri* was collected from Northern Pakistan, Fairy Meadows, 3036 m a.s.l., occupying rock crevices along a running stream. A healthy flowering plant was also collected for accurate host identification. Free hand sections of infected material and spores were mounted in lactophenol. Semi-permanent slides were prepared by cementing cover slips with nail lacquer (Dade & Gunnell 1969). Preparations were observed under a Nikon YS100 microscope and

photographed with a digipro-Labomed (USA). Spore drawings were made using a Leitz Camera Lucida (Wetzlar, Germany). Twenty-five spores were measured using a Zeiss ocular micrometer (St Albans, England). Measurements include range and arithmetic means with extremes shown in parenthesis.

Taxonomy

Puccinia cortusae Tranzschel, in Sydow & Sydow, Monogr. Uredin. (Lipsiae) 1(5): 886 (1904). Figs. 1–2

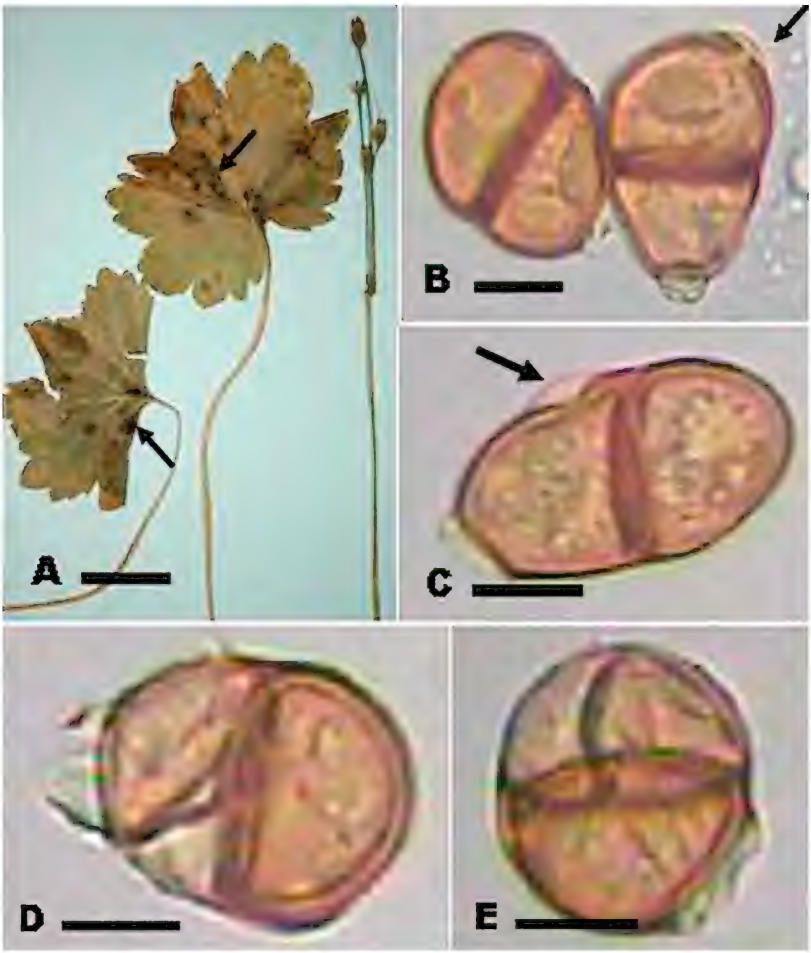

Fig. 1. *Puccinia cortusae.* A: Infected host plant, *Cortusa brotheri* (arrows showing telia along and between veins); B–C: Teliospores (arrows showing sub-apical position of germ pore in distal cell and next to septum in proximal cell with hyaline papillae); D–E: Aberrant teliospores with additional oblique and perpendicular septa. Scale bars: A = 2 cm, B–E = 10 µm.

Spermogonia, aecia and uredinia not seen. Telia hypophyllous, rarely epiphyllous, circinate, pulverulent, covered by ruptured epidermis, naked, along or between veins, 70–140 × 280–400 µm. Teliospores pale brown to chestnut brown, ellipsoid, broadly ellipsoid or obovoid, rounded or attenuated at base, slightly or not constricted at septum, chestnut brown at septum, 16–25(–31) × 24–37 µm (mean 22.2 × 29 µm); wall chestnut brown, smooth, 1–1.6 µm; germ pore one per cell, with variable position, mostly apical, sub apical or sometimes adjacent to septum in distal cell; in proximal cell mostly adjacent to septum, sometimes shifted ½ to ¾ towards hilum, germ pores with hyaline papillae; apex not thickened, rounded, sometimes conical; pedicel hyaline, deciduous, short, sometimes obliquely attached, 5–11 × 3–20 µm. One-celled teliospores and aberrant teliospores with additional oblique or perpendicular septa present but rare.

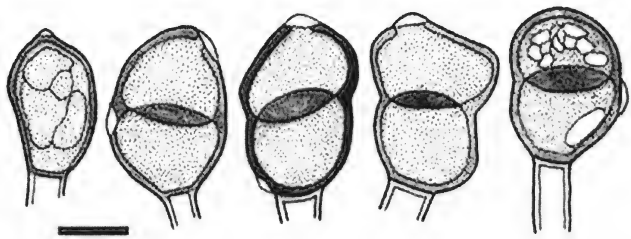

Fig. 2. Lucida drawings of *Puccinia cortusae* showing one- and two-celled teliospores. Scale bar = 10 µm.

Material examined: **PAKISTAN**, Fairy Meadows, Bial Camp, on *Cortusa brotheri* Pax ex Lipsky (*Primulaceae*), 24 July 2010, Malka Saba 07, with III stage (LAH 1147).

Comments: Among new observations made for *P. cortusae* not mentioned in the original description are the presence of both two-celled and one-celled teliospores that are broader (15–21 µm in the original). Teliospore germ pores were observed in different positions, e.g., apical, subapical, or sometimes adjacent to the upper cell septum although they were mostly observed in the lower cell adjacent to the septum or sometimes shifted half or three-quarters of the distance toward the hilum.

Puccinia cortusae is the only rust fungus reported on *Cortusa* L., and has previously been known only from the type collection on *C. matthioli* L. from the Alai Range in southern Kyrgyzstan (Sydow & Sydow 1904: "in jugo Alaico Turkestaniae"; Farr & Rossman 2011). Our collection is a new record of *P. cortusae* for southern Asia and *C. brotheri* is a new host for this rust fungus. The two hosts are closely related, with *C. brotheri* sometimes treated as a form of *C. matthioli, C. matthioli* f. *brotheri* (Pax ex Lipsky) R. Knuth.

Gäumann (1959) described *Puccinia cortusae* as a microform (telial stage only) of *P. primulae* Grev. Teliospores resemble those of *P. primulae* in shape although they differ in size, those of *P. cortusae* being longer and broader (16–25(–31) × 24–37 µm vs. 15–18 × 22–30 µm) with chestnut brown wall, variable position of germ pores and apically unthickened.

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Gymnopus fuscotramus (*Agaricales*), a new species from southern China

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Abstract — A new species, *Gymnopus fuscotramus,* is described from China. It is characterized by brown-incarnate colors in pileus and lamellae, sulcate pileus, free and distant lamellae, floccose-squamulose, mostly black stipe, well-developed black rhizomorphs, repent and diverticulate pileipellis hyphae, abundant clamp connections, diverticulate to coralloid cheilocystidia, moderately thick-walled caulocystidia with obtuse apex, dextrinoid hyphae in cortex of stipe, and gray-brown pileal and hymenophoral trama. Color images of basidiomata and microscopic elements accompany the description. *Gymnopus fuscotramus* is compared with similar species and its systematic position is also inferred using the ITS rDNA sequence data.

Key words — *Basidiomycota,* biodiversity, *Omphalotaceae,* taxonomy

Introduction

During field research on marasmioid fungi in southern China (Guangxi Zhuang Autonomous Region), the third author collected an interesting fungus which is described here as a new species of the genus *Gymnopus* (Pers.) Roussel. According to morphological characters, it belongs to section *Androsacei* (Kühner) Antonín & Noordel., which is characterized by small and marasmioid basidiomata, thin, insititious and mostly dark colored stipe, usually well-developed black rhizomorphs, dextrinoid trama of stipe and non-hymeniform pileipellis (Antonín & Noordeloos 2010). Members of this section were traditionally placed in the genus *Marasmius* (Fries 1838). Kühner (1933) described section *Androsacei* under the same genus. On the basis of

its non-hymeniform pileipellis, Antonín (1987) excluded members of the section *Androsacei* from *Marasmius,* and described a new genus *Setulipes*. Molecular phylogenetic studies published by Moncalvo et al. (2002), Mata et al. (2004) and Wilson & Desjardin (2005) showed that the type species of the genus *Setulipes*, *S. androsaceus* (L.) Antonín, belongs to the gymnopoid clade. Therefore, Noordeloos & Antonín (2008) transferred section *Androsacei* to the genus *Gymnopus*.

Materials & methods

The description of *Gymnopus fuscotramus* is based on one collection consisting of seven basidiomata, which were photographed in the field. Color codes in the macroscopic description (given in brackets) are cited according to Kornerup & Wanscher (1981). Microscopic features were observed with a light microscope (brightfield and phase contrast – PhC) under magnification up to 1500× and photographed with a digital camera. Description and images of microscopic characters were made from rehydrated specimens mounted in 2.5% potassium hydroxide (KOH) solution. Amyloidity and dextrinoidity were tested in Melzer's reagent (Erb & Matheis 1983). Basidiospore measurements were calculated from mounts of lamellae and based on calibrated digital images. A total number of 50 randomly selected basidiospores from two mature basidiomata were measured. Spore measurements (length, width) are given as: (min.) stat. min. – av. – stat. max. (max), where "min." = minimum (lowest measured value), "stat. min." = statistical minimum (arithmetic average minus two times standard deviation), "av." = arithmetic average, "stat. max." = statistical maximum (arithmetic average plus two times standard deviation), "max." = maximum (highest measured value). Standard deviation (SD) of spore length and width is also given. The length/width ratio of spores is given as the "Q" value (min. – av. – max.). The holotype is deposited in the Herbarium of Guangdong Institute of Microbiology (GDGM), while an isotype is deposited in the Croatian National Fungarium (CNF). Comparison of *Gymnopus fuscotramus* with similar taxa is based on revision of type specimens of *Marasmius nigroimplicatus* Corner (E 206719) and *M. subrigidichorda* Corner (E 206861), as well as descriptions in the following literature: Petch 1948, Singer 1976, 1989, Pegler 1986, Desjardin 1987a,b, Corner 1996, and Antonín 2007.

Genomic DNA was isolated from dried material with E.Z.N.A. forensic kit (Omega bio-tek) according to manufacturer's protocol for isolation of DNA from hair, nails and feathers. The primers ITS1F and ITS4 (White et al. 1990) were used for amplification and sequencing of ITS region, containing the ITS1, 5.8S and ITS2 regions of rDNA. PCR amplifications were performed in a total volume of 25 µl. The initial denaturation step at 95 °C for 85 s, was followed with 35 cycles of 94 °C for 35 s, 55 °C for 45 s, and 72 °C for 60 s. PCR products were subcloned into pGEM-T vector (Promega) according to the manufacturer's instructions. Three positive clones were sequenced using the pUC or T7 vector primers with the ABI BigDye Ready Reaction Kit on an ABI 3100 automated sequencer. Sequencing reads were assembled using Lasergene processing software (DNASTAR Inc., Madison, USA) and checked manually for sequencing errors. Sequence, submitted to GenBank with accession number JF303730, was compared

Figs 1–2. *Gymnopus fuscotramus* (holotype). 1. Basidiomata in situ. 2. Pileipellis (PhC). Bars: 1 = 10 mm; 2 = 10 μm.

by homology searches with known sequences using BLAST (Benson et al. 2003). ITS sequences from 25 species taken from NCBI were used for further analysis. An alignment of the sequences was performed using Clustal X (Thompson et al. 1994). Ambiguously aligned regions were determined and excluded from further analyses using the online version of the program Gblocks 0.91b, under less stringent parameters (Castresana 2000). Final alignment was 620 bp long (available upon request). Jmodeltest (Posada 2008, Guindon & Gascuel 2003) was used to select the best-fit model of nucleotide substitution. Phylogenetic analyses were performed using Bayesian MCMC, maximum parsimony (MP) and Neighbor-joining (NJ) methods. Four species of *Marasmius* were selected as outgroup taxa for rooting purposes. Bayesian inference of the phylogeny using Metropolis coupled Markov chain Monte Carlo analyses (Geyer 1991) was performed using MrBayes, version v. 3.1.2. (Ronquist & Huelsenbeck 2003), under the Hasegawa-Kishino-Yano + gamma (HKY+G) model, which incorporates different rates for transitions and transversions and rate variation across sites. MCMC sampling was performed as implemented in MrBayes with the default settings (two runs of four chains each) for 10,000 generations, with the first 10% discarded as burn-in. MrBayes was used to compute a 50% majority rule consensus of the remaining trees to obtain estimates for the posterior probabilities (PPs) of the groups. Branch lengths were computed as the mean values over the trees sampled after burn-in. MP and NJ analyses were performed in Mega version 4 (Tamura et al. 2007). Relative robustness of individual branches was estimated by bootstrapping (BS), using 1000 replicates.

Taxonomy

Gymnopus fuscotramus Mešić, Tkalčec & Chun Y. Deng, **sp. nov.** Figs 1–7

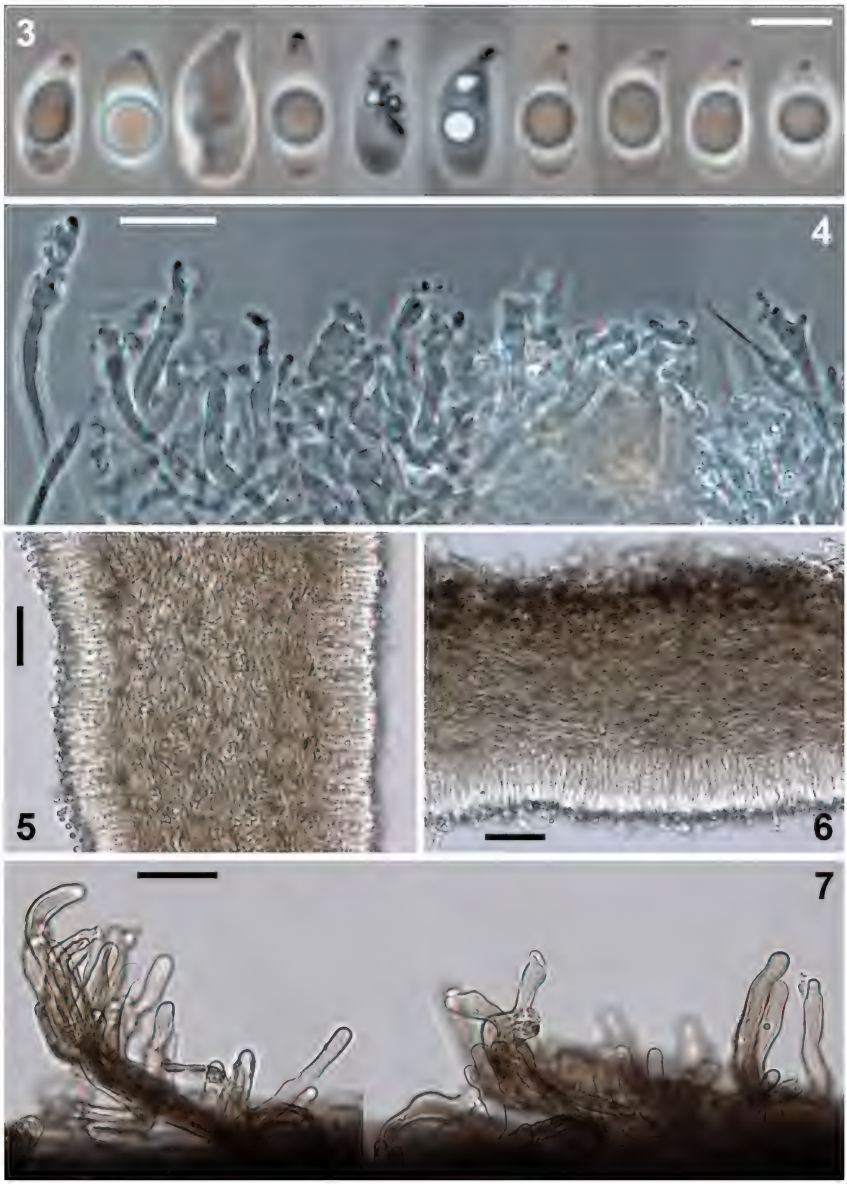
MycoBank MB 519324

Pileus 12–21 mm latus, campanulatus, sulcatus, brunneolo-incarnatus usque incarnato-brunneus. Lamellae liberae, distantes, brunneolo-incarnatae. Stipes 15–30 × 1–1.5 mm, floccoso-squamulosus, apice aurantio- usque rubro-brunneus, humilius nigro-brunneus usque niger, insiticius. Sporae (6.7–)6.8–8.2–9.6(–9.8) × (3.0–)3.0–3.7–4.4(–4.8) µm, oblongae, subcylindricae, amygdaliformes vel lacrimiformes, hyalinae. Cheilocystidia 20–45(–60) × 5–15(–25) µm, subcylindrica usque irregulariter clavata et diverticulata vel coralliformia. Pleurocystidia absentia. Caulocystidia 5–100 × 4–12 µm, plerumque cylindrica, subcylindrica vel anguste clavata, apice obtusa, crasse tunicata (0.5–2 µm). Pileipellis cutis, hyphis diverticulatis. Trama pilealis et hymenophoralis fusca. Fibulae abundantes. Hyphae in corticali stipite dextrinoideae.

Etymology: Named for the gray-brown color of its pileal and hymenophoral trama.

Holotype: CHINA, GUANGXI: Maoershan Nature Reserve, 72 km N of Guilin, 25°54'32"N, 110°27'30"E, alt. 1500 m, 29 May 2009, leg. C.Y. Deng, GDGM 26313. Isotype: CNF 1/6044.

Pileus 12–21 mm broad, campanulate, sometimes with applanate or slightly depressed center, sulcate almost to the center, weakly translucently striate when moist, hygrophanous, brownish incarnate to incarnate-brown when moist (from 10B4 to 11D5), pale incarnate on drying, with darker, mostly dark red-brown to blackish-brown center, surface dull, dry. Lamellae free (without



Figs 3–7. *Gymnopus fuscotramus* (holotype). 3. Spores (PhC). 4. Cheilocystidia (PhC). 5. Hymenophoral trama. 6. Pileal trama. 7. Caulocystidia. Bars: 3 = 5 µm; 4 = 20 µm; 5–7 = 30 µm.

a collarium or pseudocollarium), distant (L = ca. 12, l = 1), ≤2 mm broad, ventricose, reaching the margin of the pileus or almost so, sometimes slightly intervenose, brownish incarnate, with entire, concolorous edge. Stipe 15–30 × 1–1.5 mm, subcylindrical, orange- to red-brown in the upper part, downward brown-black to black, entirely pale brown floccose-squamulose, dry, hollow, insititious, arising directly from substrate (not from rhizomorphs). Context grayish-brown and very thin in pileus, whitish in stipe medulla, concolorous with surface in stipe cortex. Smell and taste not recorded. Rhizomorphs abundant, filiform, unbranched, ≤135 mm long and ≤0.5 mm thick, glabrous, black, with black to black brown inner context, hollow, apex with white globule or tapering and concolorous.

Spores [50/2/1] (6.7–)6.8–*8.2*–9.6(–9.8) × (3.0–)3.0–*3.7*–4.4(–4.8) µm, SD = 0.71 × 0.34, Q = 1.73–*2.23*–2.70, oblong to subcylindrical, in side view often amygdaliform or even lacrymoid, smooth, hyaline, thin-walled, non-amyloid, non-dextrinoid. Basidia 23–36 × 4.5–6 µm, narrowly clavate, (2)4-spored, thin-walled, hyaline, clamped. Basidioles narrowly clavate, cylindrical or fusoid. Lamellar edge sterile, composed of repent, diverticulate, hyaline to gray-brown, 1.5–8 µm broad hyphae, with clusters of cheilocystidia. Cheilocystidia 20–60 × 5–15(–25) µm, subcylindrical to irregularly clavate with irregular finger- to knob-like projections or coralloid, thin- to moderately thick-walled (≤0.8 µm thick), hyaline to pale gray-brown. Pleurocystidia absent. Hymenophoral trama irregular, composed of 1–6(–8) µm broad, thin- to moderately thick-walled (≤1 µm thick), subhyaline to pale gray-brown (gray-brown in mass) hyphae, pigment intracellular. Pileipellis a subregular cutis composed of 1.5–10 µm broad, mostly thin-walled, less frequently moderately thick-walled (≤0.8 µm thick), subhyaline to brown, mostly diverticulate hyphae, with occasional coralloid elements, dark brown pigment often coarsely encrusted. Pileal trama composed of pale gray-brown (gray-brown in mass), non-gelatinized, thin- to moderately thick-walled (≤0.8 µm thick), 1–7 µm broad hyphae, pigment intracellular. Stipitipellis a cutis of parallel, 1.8–7 µm broad, brown hyphae with intracellular, sometimes also encrusted pigment. Caulocystidia very abundant, 5–100 × 4–12 µm, mostly cylindrical, subcylindrical or narrowly clavate, with obtuse apex, sometimes diverticulate, moderately thick-walled to thick-walled [walls 0.5–2(–2.5) µm thick], sometimes with one septa, often in groups, subhyaline to brown, with intracellular, sometimes also encrusted pigment. Stipe trama composed of parallel, thin- to thick-walled (≤1.2 µm thick), 2–10 µm broad hyphae, hyaline in stipe medulla. Clamp connections present and abundant in all tissues. Chemical reactions: all parts of basidioma non-amyloid and non-dextrinoid except hyphae in cortical layer of stipe and caulocystidia, which are dextrinoid (red-brown in Melzer's reagent).

Habitat — In small clusters on a twig, in humid subtropical forest of *Fagus longipetiolata* and *Castanopsis lamontii.*

Distribution — Known only from the type locality in China.

Discussion

Gymnopus fuscotramus is characterized by a brown-incarnate colors in pileus and lamellae, sulcate pileus, free and distant lamellae, floccose-squamulose, mostly black stipe, well-developed black rhizomorphs, growing only on woody substrate (not arising from rhizomorphs), non-hymeniform pileipellis (cutis with diverticulate hyphae), abundant clamp connections, diverticulate to coralloid cheilocystidia, moderately thick-walled caulocystidia with obtuse apex, dextrinoid hyphae in cortex of stipe, and gray-brown pileal and hymenophoral trama.

Among other species in the section *Androsacei* with clamp connections, black rhizomorphs, non-glabrous stipe, and colored pileus and lamellae (not white or cream), *Marasmius bactrosporus* Singer, *M. campinaranae* Singer, and *Setulipes brevistipitatus* Antonín have (among others) much smaller basidiomata and more elongated spores. *M. nigroimplicatus* has thinner (≤0.2 mm) and minutely pubescent rhizomorphs, adnate lamellae sometimes attached to a pseudocollarium, more elongated spores, and caulocystidia with thicker walls (1.8–5 µm). *M. rigidichorda* Petch has basidiomata arising also from the rhizomorphs, adnate lamellae, white context, hyaline hymenophoral trama, and caulocystidia with pointed apex. *M. subrigidichorda* has basidiomata arising also from the rhizomorphs, lamellae attached to a pseudocollarium, longer and wider rhizomorphs (≤410 × 0.9 mm), hyaline hymenophoral trama, and caulocystidia with thicker walls (2–5 µm). *M. thiersii* Desjardin has crowded, adnate to adnexed lamellae, rare or absent rhizomorphs, and lacks cheilocystidia. *Setulipes rhizomorphicola* Antonín has much shorter (≤3 mm) and eccentric stipe arising from rhizomorphs, adnate lamellae, and longer spores.

According to morphological characters, *Gymnopus fuscotramus* belongs to section *Androsacei.* However, our phylogenetic analysis using only ITS rDNA sequences do not place our taxon on the same clade with the two sequenced species from that section, *G. androsaceus* (L.) J.L. Mata & R.H. Petersen and *G. quercophilus* (Pouzar) Antonín & Noordel. However, *G. fuscotramus* is placed close to *G. peronatus* (Bolton) Gray (sect. *Vestipedes*). To further clarify phylogenetic relationships between *G. fuscotramus* and other *Gymnopus* species (especially from sect. *Androsacei*), more species (reliably identified) and more DNA sequences should be included in analyses. Our gymnopoid clade also includes *Marasmiellus* and *Rhodocollybia* species, which is in accordance with results of Moncalvo et al. (2002), Mata et al. (2004) and Wilson

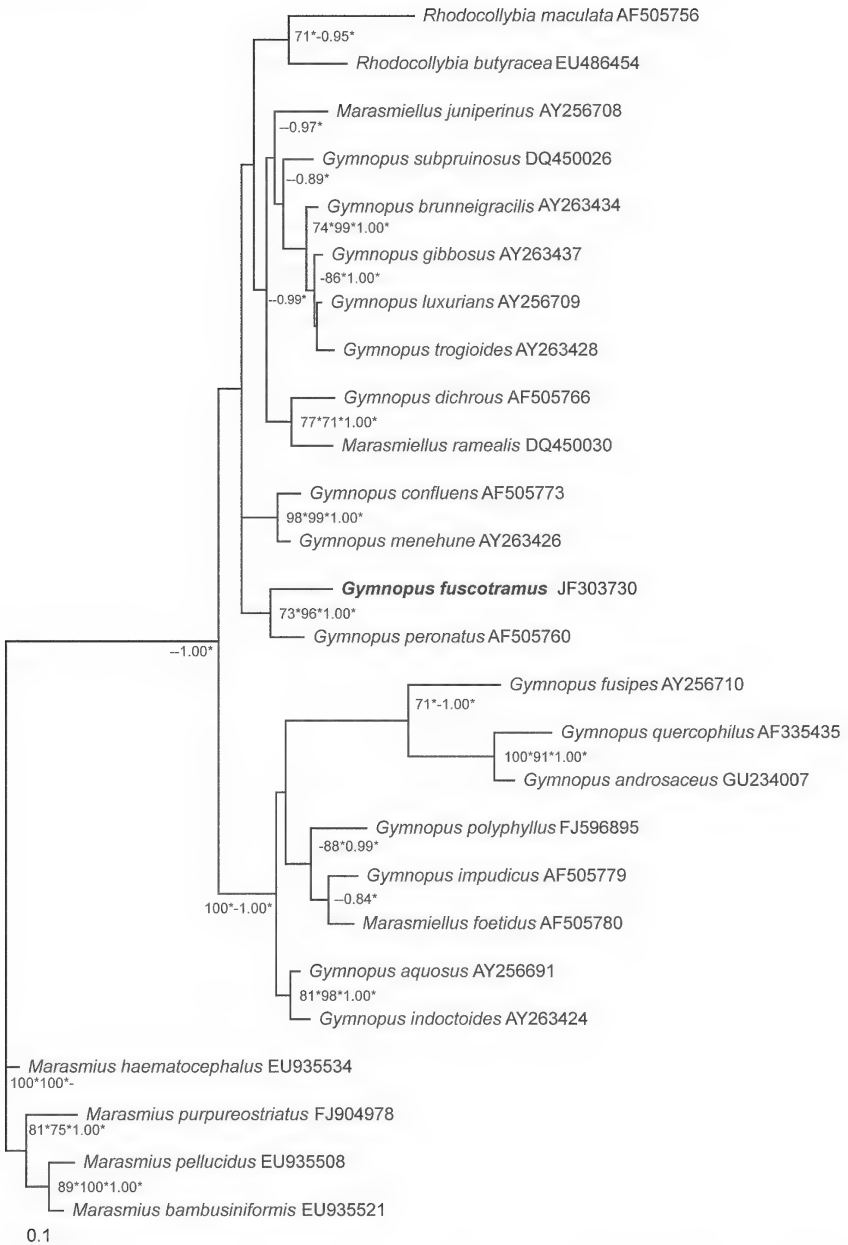


Fig. 8. Phylogenetic tree based on the complete ITS rDNA region (ITS1, 5.8S rDNA, and ITS2), showing mean branch lengths of a 50 % majority-rule consensus tree from a Bayesian MCMC analysis. NJ, MP bootstrap values (>70%), and Bayesian PP values are given above nodes.

& Desjardin (2005). All these results indicate that *Gymnopus* and *Marasmiellus* (as conceived recently) are polyphyletic and that new taxonomic concepts inferred from DNA sequences should be proposed. However, since past phylogenetic analysis included only a small number of species from these two genera, further taxonomic solutions should be based on more extensive phylogenetic research with more species and DNA sequences included.

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Biogeographical patterns in pyrenomycetous fungi and their taxonomy. 2. Additions to the Grayan disjunction

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Abstract — We report additional examples of pyrenomycetous fungi that display the biogeographical pattern known as the Grayan disjunction. *Daldinia grayana* and *Diatrypella informis* are described as new species, and *Nemania pseudoillita* is proposed as a new combination.

Key words — *Ascomycota*, biogeography, new species

Introduction

Additional collecting trips and visits to several different herbaria have revealed a number of specimens that warranted a re-identification. The data available on their distribution indicate that they display the biogeographical pattern known as the Grayan disjunction, which was discussed in a previous paper (Vasilyeva & Stephenson 2010).

Two types of this distribution pattern have been observed in pyrenomycetous fungi—vicariance and non-vicariance. In addition, there are two other groups of fungi restricted to either eastern Asia or eastern North America that do not have close relatives in the other region. Although these two groups of species are seemingly pertinent to the two regions under consideration, the biogeographical patterns they exhibit have different explanations, which will be considered in a forthcoming paper. The purpose of the present paper is to provide additional information relating to the Grayan disjunction in pyrenomycetes and to discuss certain aspects of this pattern.

Materials & methods

The specimens included in this study are deposited in FH, MICH, and VLA. The maps used as the basis for illustrations were taken from the web sites commons.wikimedia.org

(Figs 1–3) and www.paleoportal.org (Fig. 4); all maps were modified to show our data. Photographs of ascomata were obtained using a Nikon D40x digital camera.

Examples of the non-vicariance pattern

Daldinia grayana Lar.N. Vassiljeva & S.L. Stephenson, **sp. nov.** Fig. 1

MycoBank MB 561654

Stromata solitaria vel aggregata et confluentia, sessilia vel breve stipitata, 0.5–2.5 cm diam. × 0.8–2.5 cm alta, atro-lateritia, demum denigrata et laccata, rugosa, epapillata et sine tumulis peritheciorum, pigmenti griseo- vel veriduli-olivacei, interdum atro-gresei vel plumbei in KOH dissoluti; textura sub peritheciis zonis alternantibus composita, zonis fuscis 0.3–0.6 mm crassis et zonis cremeo-fuscis 0.1–0.3 mm crassis praedita. Perithecia tubularia, 0.1–0.2 mm diam. × 0.4-0.8 mm alta. Asci partibus sporiferis 80–100 × 7–8 µm, stipitibus 80–100 µm longitudine, annulo apicali in liquore iodato Melzeri cyanescente, discoideo, 3–4 × 0.8–1 µm. Ascosporae brunneae, unicellulares, brevi- et late-ellipsoideae vel anguste-ellipsoideae, apicibus anguste-rotundatis, (10–)12–14(–16) × 6–7.5 µm, rima germinativa recta longa praeditae; perisporium in KOH dehiscens, leve.

Type: Russia: Primorsky Territory, Lazo Nature Reserve, on *Alnus hirsuta* (Spach) Turcz. ex Rupr. (*Betulaceae*), 4 Aug 1986, *L. Vasilyeva* (**Holotype** VLA P-2547).

Stromata solitary to aggregated and confluent, sessile or short stipitate, 0.5–2.5 cm diam., 0.8–2.5 cm high, dark reddish brown, blackened and varnished in age, wrinkled, lacking papillae and perithecial mounds, KOH-extractable pigments gray-olivaceous or greenish-olivaceous, sometimes dark-gray or plumbeous; tissue below the perithecial layer composed of alternating zones, the darker zones dark brown, 0.3–0.6 mm thick, the lighter zones creamy-brown, 0.1–0.3 mm thick. Perithecia tubular, 0.1–0.2 mm diam. × 0.4–0.8 mm high. Asci in the spore-bearing part 80–100 × 7–8 µm, the stipe 80–100 µm long, with apical ring bluing in Melzer's iodine reagent, discoid, 3–4 × 0.8–1 µm. Ascospores brown, unicellular, variable in shape, broadly ellipsoid to more narrow-ellipsoid, (10–)12–14(–16) × 6–7.5 µm, with a straight germ slit spore-length; perispore dehiscent in 10% KOH, smooth.

Additional specimens examined: **China: Heilongjiang Province**, Raohe, on *Alnus hirsuta*, 7 Aug 2004, *L. Vasilyeva* (VLA P-2551). **Canada: Nova Scotia**, Salmon River, 14 Jul 1931, *L.E. Wehmeyer* (MICH 71984, as *Daldinia concentrica*). **USA: New York**, Ithaca, Aug 1902, *C.H. Kauffman* (MICH 71986, as *Daldinia concentrica*; substrate not indicated); **Vermont**, Pawlett, on *Betula papyrifera* Marshall (*Betulaceae*), *C.W. Dodge & D.H. Linder* (FH, as *Daldinia concentrica*); **Michigan**, Vermilion Point, 30 Jul 1914, *A.H. Povah* (MICH 71987 & 71988, as *Daldinia concentrica*); Rock River, on *Alnus* sp., 24 Aug 1927, *A.H. Povah* (MICH 71989, as *Daldinia concentrica*); SE of Stockbridge, on dead wood, 20 Aug 1928, *E. Whitney & B.B. Kanouse* (MICH 71992, as *Daldinia concentrica*); Rock Harbor, on *Betula papyrifera*, 20 Apr 1930, *A.H. Povah* (MICH 71990, as *Daldinia concentrica*); Todd Harbor, on *Alnus* sp., 24 Aug 1930, *A.H. Povah* (MICH 71985, as *Daldinia concentrica*); McCargoe Cove, on *Alnus* sp., 1930 (date not indicated), *A.H. Povah* (MICH 71982, as *Daldinia concentrica*); Conway Lake Road, on *Betula alleghaniensis* Britton, 12 Aug 1970, *K.A. Harrison* (MICH 71983, as *Daldinia concentrica*); Pine Lake, on *Betula* sp., 23 Jul 1971, *K.A. Harrison* (MICH 71991, as

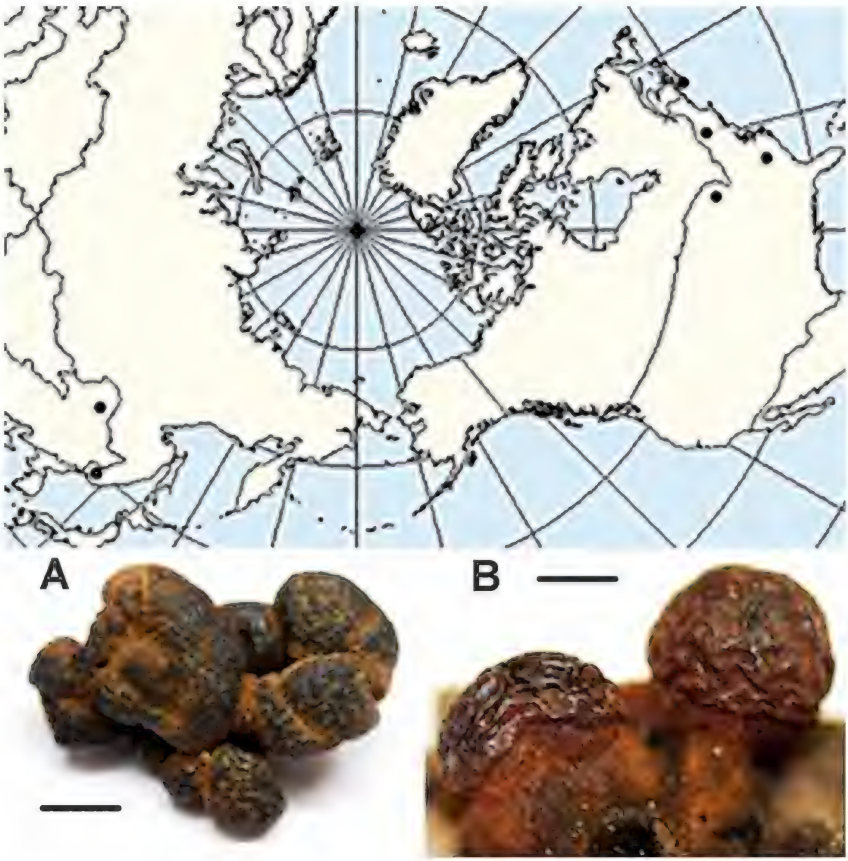

FIG. 1. Approximate biogeographical distribution of *Daldinia grayana*. Scale bars: A = 10 mm, B = 5 mm.

Daldinia concentrica); **TENNESSEE**, Smoky Mountains National Park, Newfound Gap, on *Betula* sp., 13 Aug 1968, *D.A. Reid* (K, as *Daldinia vernicosa*).

COMMENTS–The appearance of *Daldinia grayana* is similar to that of *D. gelatinosa* Y.M. Ju et al. as the latter is illustrated (Ju et al. 1997, FIG. 44) and described (Ju et al., 1997, p. 270: "Stromata... sessile or short stipitate, solitary or aggregated, wrinkled, ...surface dark brick, blackened and varnished in age..."). It is also noteworthy that *D. gelatinosa* appears to have a similar distribution in eastern North America and is reported from Ontario (Canada), Idaho, and New Hampshire. The latter record is associated with *Betula alleghaniensis*, as is one record of *D. grayana*.

The resemblance of *Daldinia gelatinosa* to *D. grayana* is reinforced by the fact that one piece of the exsiccate of '*Daldinia concentrica*' in Ellis & Everhart's

"Fungi Columbiani 213" was identified as *D. gelatinosa* (Ju et al. 1997: WSP 1781), whereas we identify another piece (MICH 71993) as *Daldinia grayana*. Except for this questionable material, *D. gelatinosa* differs from *D. grayana* in having dark-livid KOH-extractable pigments rather than gray- or greenish-olivaceous pigments, as well as in the nature of the inner tissue of the stromata. This tissue in *D. gelatinosa* is similar to that of *D. fissa* Lloyd in having the alternating zones of dark brown and white, which are initially gelatinous but disintegrate and become loculate when dry. In contrast, *D. grayana* has stromata that are solid inside, and their alternating zones are only darker and lighter tinges of brown, although sometimes the lighter zones are cream-colored.

Based on the abundant material from Michigan, *Daldinia grayana* might be more of a northern or high elevation species (cf. the record from Newfound Gap) than *D. gelatinosa*; in this respect, *D. grayana* is similar to *D. loculata* (Lév.) Sacc., which occurs on birch (*Betula* spp.) in the more northern parts of the Holarctic; when *D. loculata* is found in more southern regions, it occurs primarily in the mountains.

In terms of the substrate preferences (*Alnus* spp., *Betula* spp.), *Daldinia grayana* should be also compared with *D. decipiens* Wollw. & M. Stadler, which is known to occur on *Betula* spp. in Europe (Stadler et al. 2001b), and *D. petriniae* Y.M. Ju et al. described on *Alnus* also from Europe (Ju et al. 1997) but later found to be circumpolar (Stadler et al. 2001b, p. 175). Only one locality for *D. petriniae* in the United States is from the Pacific coast region (Stadler et al. 2001a, c). This species differs from *D. grayana* in having larger, more fragile stromata, livid purple or dark livid pigments, and a finely papillate stromatal surface (similar to the condition illustrated by *D. childiae* J.D. Rogers & Y.M. Ju as seen on the website http://mycology.sinica.edu.tw/Xylariaceae). We have a specimen of *D. petriniae* on *Alnus hirsuta* from China (Heilongjiang Province) collected in 2004. The pigments in its stromata display pale-vinaceous-grey pigment in 10% KOH instead of livid purple or dark livid, but it is the same portion of the color spectrum in contrast to greenish-olivaceous tints observed in *D. grayana*.

Daldinia decipiens is similar to *D. petriniae* in having purple, dark livid or vinaceous purple KOH-extractable pigments (Stadler et al. 2001b). Morphologically, these two species are sometimes difficult to distinguish, although *D. decipiens* appears to have larger ascospores; it also differs from *D. petriniae* in the smaller, often stipitate, stromata with a smooth surface and more solid inner tissue with brown zones of very close tinges. In addition, the stromata of *D. decipiens* are reddish brown, especially the stalks, whereas those in *D. petriniae* are mostly brown vinaceous. The color is shared by *D. decipiens* with *D. grayana*, but the stromatal pigments and consistency easily distinguish them.

Diatrypella informis Ellis & Everh. ex Lar.N. Vassiljeva & S.L. Stephenson, **sp. nov.**

MycoBank MB 561655

Stromata solitaria vel aggregata, e cortice erumpentia, linea nigra substrato circumdata, verrucaeformia, plano-convexa, suborbicularia vel ellipsoideae, 4–10 × 1.5–4 mm, laeviuscula vel leviter rugosa, nigra, intus albida. Perithecia subglobosa, 0.1–0.2 mm diam., profunde immersa, cum ostioli integri, umbilicati vel leviter papillati, Asci longe ellipsoidei vel clavati, polyspori, aparaphysati, partibus sporiferis 35–60 × 5–7 µm, stipitibus ad 75 µm longitudinus, tunica apice incrassata, annulo apicali nullis. Ascosporae allantoideae, unicellulares,brunneolae vel flavidae, 4–6 × 1–1.2 µm.

Type: Ellis & Everhart's North American Fungi N 2530: "*Diatrypella informis* E. & E. n. sp., on dead *Carpinus*, London, Canada, Apr. 1890, J. Dearness". [**Holotype** BPI].

Stromata usually densely scattered over the branches of the host tree, surrounded by a black zone, erumpent from the bark with a black, robust, rounded or ellipsoid, convex, smooth or somewhat wrinkled ectostromatic disc 4–10 × 1.5–4 mm in diam. Perithecia subglobose, 0.1–0.2 mm diam., deeply immersed, the tissue above perithecial layer white, ostioles umbilicate or slightly papillate, non-sulcate. Asci long ellipsoid or slightly clavate, polysporous, in the spore-bearing portion 35–60 × 5–7 µm, stalks up to 75 µm, wall thickened at the apex, without an apical ring and paraphyses. Ascospores unicellular, allantoid, brownish yellow when crowded in asci, pale yellow when scattered, 4–6 × 1–1.2 µm.

Additional specimens examined: **RUSSIA: Primorsky Territory**, Vladivostok vicinity, on *Carpinus cordata* Blume (*Corylaceae*), 6 Jun 1958, *I. Bunkina* (VLA P-1975); 11 Oct 1991, *L. Vasilyeva* (VLA P-1978 & P-2129); 5 Oct 2006, *L. Vasilyeva* (VLA P-1108); Sikhote-Alin Nature Reserve, on *Carpinus cordata*, 24 Oct 1985, *L. Vasilyeva* (VLA P-2126); Kedrovaya Pad Biosphere Reserve, on *Carpinus cordata*, 4 Oct 1987 & 25 Oct 1987, *L. Vasilyeva* (VLA P-2127 & P-2128); Ussuriysk Nature Reserve, on *Carpinus cordata*, 19 Aug 1989, *L. Vasilyeva* (VLA P-2265); Anisimovka, on *Carpinus cordata*, 20 Sep 1996, *L. Vasilyeva* (VLA P-2130).

Comments– *Diatrypella informis* was illustrated in the previously published paper on biogeographic patterns in pyrenomycetous fungi (Vasilyeva & Stephenson 2010), but its name was never validly published. Therefore, a Latin description is provided herein to validate the name.

The rather large robust stromata and non-sulcate ostioles of *D. informis* are similar to those found in *D. verruciformis* (Ehrh.) Nitschke, which differs by possessing larger asci (120–200 × 8–12 µm: Nitschke 1867) and longer ascospores (6–8 µm long). The 4–6 µm range in ascospore length is used to segregate many species in the *Diatrypaceae*; examples include *Diatrype hypoxyloides* De Not., *Diatrypella decorata* Nitschke, *Eutypa mela* (Schwein.) Cooke, *Eutypella kochiana* Rehm. The regularly repetitive size ranges in different genera of the family are always very helpful in delimiting certain species within these families.

Examples of the vicariance pattern

Hypoxylon dearnessii and *H. massulatum*

Collections carried out in the vicinity of the University of Michigan Biological Station (UMBS, located near Douglas Lake, Michigan) in 2010 revealed a *Hypoxylon* species with rather variably shaped, often undulate stromata. This species was associated with *Acer rubrum*. Some of its superficial features suggested *Hypoxylon dearnessii* (Ju & Rogers 1996: Figs. 11A–B), recorded from maples only in eastern temperate North America and originally described from the vicinity of the city of London (Ontario, Canada) in the same general Great Lakes region as the UMBS area.

The KOH-extractable pigment in the Michigan specimen was the color of sienna (as in *H. dearnessii*), and the ascospores exhibited a similar size range (9–11.5 × 4.5–6 µm), although with a slightly larger range in length (9–12.5 × 4.5–5.5 µm) and with many more obovoid with one end narrowly rounded and the other broadly rounded, as in *H. fuscopurpureum* (Schwein.) M.A. Curtis. *Hypoxylon fuscopurpureum* differs in having greenish-olivaceous pigments and larger ascospores, but it and the Michigan specimen both have perispores that are indehiscent in 10% KOH. The perispore is important in delimiting *Hypoxylon* species and, in fact, prevents our assigning the Michigan specimen to *H. dearnessii*, which has a dehiscent perispore. Nonetheless, the specimen is provisionally identified as *H. dearnessii* (VLA P–2519) and is accessible for future comparative studies.

Hypoxylon dearnessii is known from Canada (Ontario, Quebec) and the United States (Colorado, Maine, New York) (Ju & Rogers 1996; Stadler et al. 2008). Specimens deposited in the Farlow Herbarium (all as '*Hypoxylon rubiginosum*'), were collected in Connecticut, Massachusetts, New Hampshire, Rhode Island, and Vermont, mostly on *Acer rubrum*. The eastern North American temperate zone is home to *H. dearnessii*, but there are some collections from eastern Asia that resemble this species. Especially suggestive is a specimen collected in 2000 on *Acer ukurunduense* from the Big Khekhtsir Nature Reserve (Khabarovsk Territory, Russia), because its stromata have the same shape as *H. dearnessii*, and the substrate was maple. However, specimens with the same type of stromata were collected later on *Ulmus* spp. in the Primorsky Territory (Russia) and in Heilongjiang Province (China).

A close examination of the stromata of specimens on *Acer ukurunduense* and *Ulmus* spp. showed their KOH-extractable pigment to be bright red instead of sienna, and they were also found to have smaller ascospores (mostly 7.5–8.5 × 3.8–5 µm) with an indehiscent perispore. Further comparisons showed that these stromata are very similar to those in *Hypoxylon massulatum* described from *Alnus hirsuta* (Vasilyeva 1998).

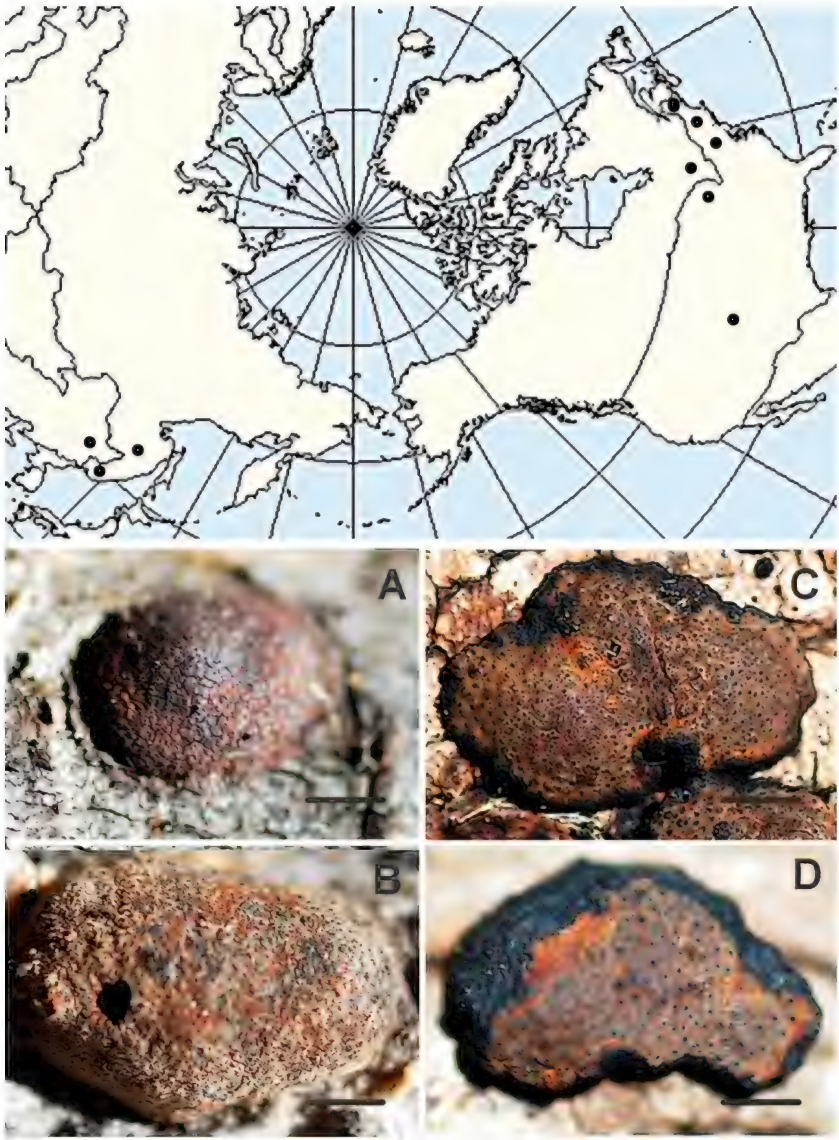

FIG. 2. Approximate biogeographical distribution of *Hypoxylon massulatum* (left) and *H. dearnessii* (right). A–B. *H. massulatum* on *Alnus* (A) and *Ulmus* (B). C–D. *H. dearnessii* on *Acer rubrum* from University of Michigan Biological Station area. Scale bars: A–B = 15 mm, C–D = 10 mm.

Hypoxylon massulatum has been considered as a synonym of *H. howeanum* Peck (Ju et al. 2004), probably because of the similar ascospore size and

stromatal pigments, but its resemblance to *H. dearnessii* (also acknowledged as showing affinities to *H. howeanum*: Stadler et al. 2008) is more evident. *Hypoxylon howeanum* has mostly globose stromata, which are sometimes rather large (up to 2 cm), brightly orange and very smooth at the surface and silky brown inside.

Hypoxylon dearnessii Y.M. Ju & J.D. Rogers, Mycol. Mem. 20: 106 (1996).

Specimens examined: **USA**: **Michigan**, Cheboygan Co., Douglas Lake, University of Michigan Biological Station, on *Acer rubrum* L. (*Aceraceae*), 12 Aug 2010, *L. Vasilyeva* (VLA P-2519); **Connecticut**, New Haven, on *Acer* sp., about 1888, *R. Thaxter* (FH, as *Hypoxylon rubiginosum*); **Massachusetts**, Waverly, on *Acer rubrum*, 23 Apr 1892, *R. Thaxter* (FH); Medford, Middlesex Fells, 13 Oct 1935, *G. Darker* 5479 (FH, as *Hypoxylon rubiginosum*); **Vermont**, Middlebury, Great Swamp, 26 Nov 1896, *E. Burt* (FH, as *Hypoxylon rubiginosum*); **New Hampshire**, near Sawyer's River, on *Acer* sp., Aug 1901, *R. Thaxter* 7112 (FH, as *Hypoxylon rubiginosum*); Chocorua, on *Acer rubrum*, 9 Aug 1907, *W. Farlow* (FH, as *Hypoxylon rubiginosum*); **Rhode Island**, Glochester, substrate not indicated, 6 Jun 1922, *D. Linder & S. Cook* (FH, as *Hypoxylon rubiginosum*).

Hypoxylon massulatum Lar.N. Vassiljeva, Nizshie Rasteniya, Griby i Mokhoobraznye Dalnego Vostoka Rossii, Griby 4: 182 (1998)

Stromata pulvinate, sometimes convex on top, rounded, ellipsoid or irregular, often with crenate margins, 0.4-1 cm diam., surface dark brick, bright orange or ochre; bright red granules immediately beneath surface, with KOH-extractable pigments orange-red. Perithecia ovoid, 200–300 µm diam., ostioles umbilicate. Asci cylindrical, the spore-bearing portion 45–50 × 5–7 µm, the stipes 30-40 µm long, with discoid apical ring bluing in Melzer's iodine reagent. Ascospores one-celled, ellipsoid, pale brown, 7.5–9(–10) × (3.5–)3.8–5 µm, with straight germ slit spore length; perispore indehiscent in 10 % KOH, smooth.

Specimens examined: **RUSSIA**: **Khabarovsk Territory**, Big Khekhtsir Reserve, on *Acer ukurunduense* Trautv. & C.A. Mey., 20 Jun 2000, *L. Vasilyeva* (VLA P-937); **Primorsky Territory**, Nadezhdino District, Sirenevka, on *Alnus hirsuta*, 29 Aug 1991, *L. Vasilyeva* (VLA P-2511); Khanka Nature Reserve, on *Ulmus* sp., 21 Jun 2003, *L. Vasilyeva*, P-936. **CHINA**: **Heilongjiang Province**, Xingkaihu Nature Reserve, on *Ulmus* sp., 1 Sept 2003, L. Vasilyeva (VLA P-1623).

Nemania illita and *N. pseudoillita*

The genus *Nemania* Gray has received attention from a number of mycologists who work with pyrenomycetous fungi (Granmo et al. 1999, Ju & Rogers 2002) but remains rather difficult taxonomically. Seven species in this genus were reported previously from the Russian Far East (Vasilyeva 1998) but as members of the genus *Hypoxylon* Bull.

Hypoxylon pseudoillitum was described as differing from *H. illitum* (Schwein.) M.A. Curtis in having larger ascospores (12-16 against 9-12 µm). The same difference exists between many *Hypoxylon* species (Vasilyeva 1983, 1985). Their stromata also differ (Fig. 3), although it is difficult to differentiate

these species in keys and descriptions. Most probably it is possible to count the number of papilla per unit of surface area, but this feature was usually not taken into account. A new combination for and English description of *H. pseudoillitum* are provided below.

Nemania pseudoillita (Lar.N. Vassiljeva) Lar.N. Vassiljeva & S.L. Stephenson, **comb. nov.** FIG. 3

MYCOBANK MB 561656

≡ *Hypoxylon pseudoillitum* Lar.N. Vassiljeva, Nizshie Rasteniya, Griby i Mokhoobraznye Dalnego Vostoka Rossii, Griby 4: 190 (1998)

Stromata effuse-pulvinate, plane, with a densely and finely papillate surface, brownish, becoming darker with age; perithecia spherical, 600–800 μm diam.

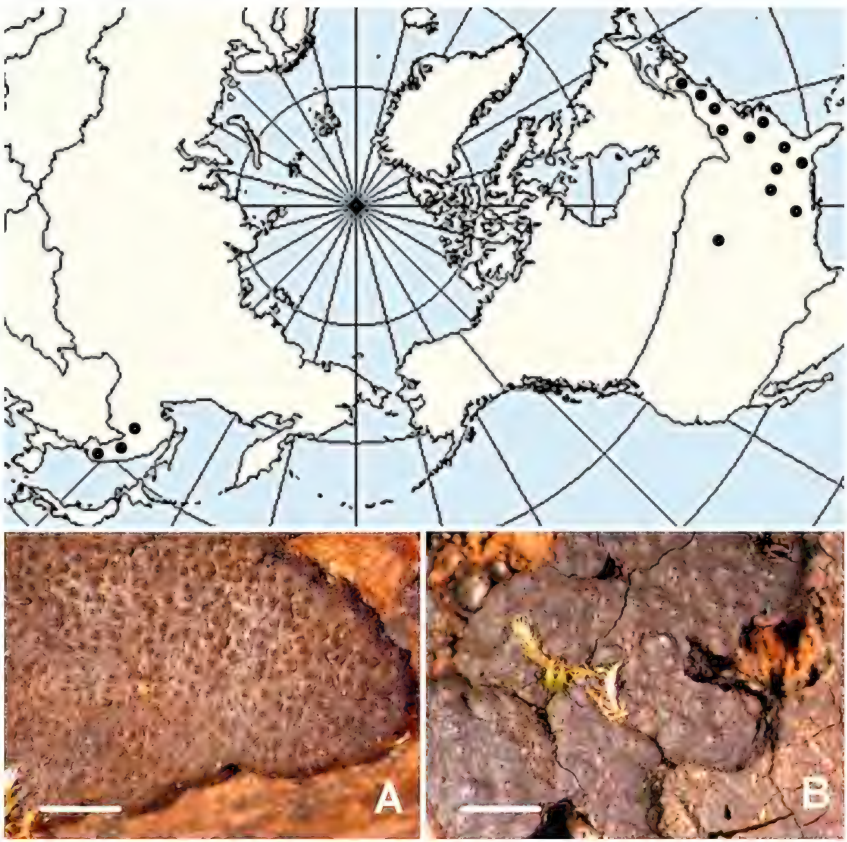

FIG. 3. Approximate biogeographical distribution of *Nemania pseudoillita* (left) and *N. illita* (right). North American localities listed by Miller (1961) include Alabama, Georgia, Kentucky, Louisiana, Maine, Maryland, Missouri, Nebraska, New Hampshire, New York, North Carolina, Pennsylvania, and Virginia in United States. Scale bar = 5 mm.

Asci cylindrical, the spore-bearing portion 80–90 × 5–7 µm, the stipes 40–50 µm long, with apical ring bluing in Melzer's iodine reagent, cubiform, 2 × 2 µm. Ascospores one-celled, diamond- or wedge-shaped, very light brown, almost hyaline, brownish in mass, (11.5–)13–15(–16.5) × (2.5–)3–4.5 µm, with a ventral germ slit.

Specimens examined: **RUSSIA: Khabarovsk Territory**, Big Khekhtsir Nature Reserve, on wood, 16 Sept 1981, *L. Vasilyeva* (VLA P-1842); **Primorsky Territory**, Lazo Nature Reserve, on wood, 26 Jul 1986, *L. Vasilyeva* (VLA P-1378); Kedrovaya Pad Reserve, on wood, 28 Oct 1987, *L. Vasilyeva* (VLA P-1380); Ussuriysk Nature Reserve, on wood, 28 Aug. 1989, *L. Vasilyeva* (VLA P-194); Vladivostok vicinity, on wood, 25 May 2003, *L. Vasilyeva* (VLA P-1379).

Discussion

We did not discuss the Grayan disjunction in pyrenomycetous fungi as part of an overview of biogeographical problems in our previous paper (Vasilyeva & Stephenson 2010). Especially interesting in this context is the vicarious pattern. The occurrence of the same species in two widely separate regions of eastern Asia and eastern North America could be attributed to the persistence of Tertiary relics following continental fragmentation, but the occurrence of intercontinental vicarious pairs of species (or varieties) is not so easily understood.

Vicarious taxa usually have very minor morphological differences (e.g., ascospore size), and it is difficult to explain why *Biscogniauxia maritima* Lar.N. Vassiljeva (ascospores 13–16 µm long) occurs only in the southern portion of the Russian Far East, north-east China, Japan, and Korea, whereas *B. atropunctata* (Schwein.) Pouzar (ascospores 24–33 µm long) is restricted to eastern North America. Both species occur on *Quercus* spp., and the ascospore size difference seems unlikely to have adaptive significance. We cannot identify any association between the environmental conditions in the two regions and the observed differences. Moreover, in some vicarious pairs, for example *Nemania illita* and *N. pseudoillita* (see above) or *Hypoxylon notatum* Berk. & M.A. Curtis and *H. ulmophilum* Lar.N. Vassilyeva (Vasilyeva & Stephenson 2010), the species with larger ascospores were found in East Asia. In the latter case, the substrate restriction of *H. notatum* and *H. ulmophilum* to *Quercus* spp. and *Ulmus* spp., respectively, also appears very strange, since representatives of both host genera are abundant in the two disjunctive areas. Although both species have plentiful host resources, they do not seem to jump from one host genus to another in eastern Asia or eastern North America.

Despite their close similarity, vicarious taxa cannot be ancestors and descendants to each other, since, if such is the case, there should be some common area of speciation. And even if such an area existed and became fragmented later, it is inexplicable how such fragmentation has so strictly

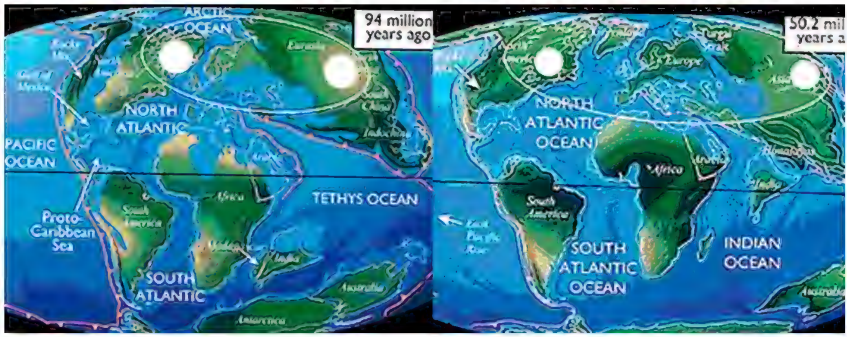

FIG. 4. The position of continents during the Cretaceous (A) and Tertiary (B) periods. White circles indicate the present landmasses of eastern Asia and eastern North America; it is evident that the original isolation of these regions has increased over time.

divided the populations in such a way that all 'ancestors' (with the same ascospore size) appear to be confined to either Asia or North America, whereas all 'descendants' occur on the other region. An additional hypothesis to account for their origin might suggest that only one species existed before continent fragmentation, and the second species evolved as a deviation of the first after long period of isolation of the two regions. However, once again it is not clear why the ancestral species disappeared completely from one fragment of the disjunctive region.

The most probable explanation of the vicariance pattern might be attributed to the position of continents during the Cretaceous and Tertiary periods (FIG. 4). Even during these earlier times, the present land masses of East Asia and eastern North America were widely separated. Therefore, the isolation of vicarious taxa was an original occurrence that only increased over time. The vicarious taxa may have been peripheral populations of some species otherwise widely distributed over Laurasia (the northern part of Pangaea).

It is particularly common for isolated populations at the margins of a range for a given species to exhibit certain distinct features, and clearly distinct peripheral populations are often observed on islands (Ridley 2003). During the Tertiary, the last phase of the breakup of Pangaea, widely separated areas with their own distinctive populations would have became biogeographical 'islands', and the vicarious taxa that exist today may simply represent fragments of the same ancestral species.

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Lignincola conchicola from palms with a key to the species of *Lignincola*

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Abstract — During studies of palm fungi, a new *Lignincola* species was found in Thailand. It differs from other members of the genus in ascospore dimensions and the occurrence of its ascomata on the adhesive pad of a marine invertebrate. *Lignincola conchicola* is described and illustrated, and a key to *Lignincola* species is provided. The palm *Phoenix* is a new substratum for marine fungi.

Key words — *Halosphaeriaceae*, mangrove, taxonomy, tropic

Introduction

During research on palm fungi in Thailand (Pinnoi et al. 2004, 2006, 2009, 2010, Pilantanapak et al. 2005, Pinruan et al. 2004a,b, 2007, 2010a,b, Rungjindamai et al. 2008, Liu et al. 2010) a new taxon collected on *Phoenix paludosa* in Ranong mangrove, was discovered. The taxon is characteristic of *Lignincola* (*Halosphaeriaceae*).

Höhnk (1955) introduced *Lignincola* for a single species *L. laevis* Höhnk, which was characterized by black perithecioid ascomata, persistent clavate to fusiform, thin-walled asci lacking an apical apparatus, and 1-septate hyaline ascospores lacking appendages. The genus differs from *Aniptodera*, *Halosarpheia,* and *Phaeonectriella* in lacking appendaged ascospores (Pang et al. 2003a,b, Jones et al. 2009). Three other *Lignincola* species have been described. One, initially described as *Gnomonia longirostris* Cribb & J.W. Cribb (Cribb & Cribb 1956), was later transferred to *Lignincola* by Kohlmeyer (1984). Pang et al. (2003a) subsequently transferred the species to a new genus *Neptunella*

based on ultrastructural and molecular evidence. Asci in *N. longirostris* have an apical pore, retraction of the plasmalemma, and ascospores with a thin mucilaginous sheath (Pang et al. 2003a). A second species, *Lignincola tropica* Kohlm., was described from mangrove wood (Kohlmeyer 1984) and has larger ascospores than *L. laevis*. The identity of this species is confused as initially the asci were described as possessing an apical pore, but subsequently Kohlmeyer & Volkmann-Kohlmeyer (1988) revised the description by stating that there was no pore to the ascus. In addition, sequence data show that collections with asci with an apical pore do not belong in *Lignincola* (Pang unpublished data). A third species, *L. nypae* K.D. Hyde & Alias (Hyde et al. 1999), was described from the brackish water palm *Nypa fruticans* and has clavate asci with a refractive apical thickening with a pore and cylindrical ascospores, features not found in *Lignincola*. A molecular study is required to resolve the placement of both *L. tropica* and *L. nypae*.

The new species *Lignincola conchicola* is described and illustrated and a key to *Lignincola* species is provided.

Materials & methods

Palm fronds were collected from Ranong mangrove, Ranong Province, Thailand. Samples were processed and examined following the methods outlined by Hyde et al. (2000) and Taylor & Hyde (2003). Several unsuccessful attempts were made to isolate and grow the fungus from single ascospores. Morphological measurements and photomicrographs were made according to Liu et al. (2010). The holotype is deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand.

Taxonomy

Lignincola conchicola J.K. Liu, E.B.G. Jones & K.D. Hyde, **sp. nov.** PLATE 1

MYCOBANK: MB 561207

Ascomata superficialia, 120–160 µm alta, 50–75 µm diametro globosa, subglobosa vel ellipsoidea, uniloculata, atro-brunnaea vel nigra. Asci 50–75(–90) × 13–16 µm, clavati vel cylindricae-clavati, unitunicati, 8-spori. Ascosporae 10.5–16 × 5–7 µm, hyalinae, ellipsoideae vel fusiformae, bicellulares, non-appendiculatae.

TYPE: Thailand, Ranong Province, Ranong mangrove, on adhesive disc of an invertebrate on submerged fronds of *Phoenix paludosa* Roxb. (*Arecaceae*), 26 January 2010, E.B.G. Jones and J.K. Liu, JKA0027 (MFLU11 0032 **holotype**).

ETYMOLOGY: Referring to its habitat on the adhesive disc of an invertebrate.

Ascomata 120–160 × 50–75 µm, superficial, globose, subglobose or ellipsoidal, uniloculate, dark brown to black, rounded at the base and developing on the adhesive pad of a marine invertebrate (Plate 1 A–B). Peridium 12–22 µm thick, membranous, comprising several layers, the outer stratum of 1–3 layers comprising dark brown, thick-walled cells, the inner 3–5 layers, textura prismatica comprising hyaline, thick-walled cells. Paraphyses and catenophyses

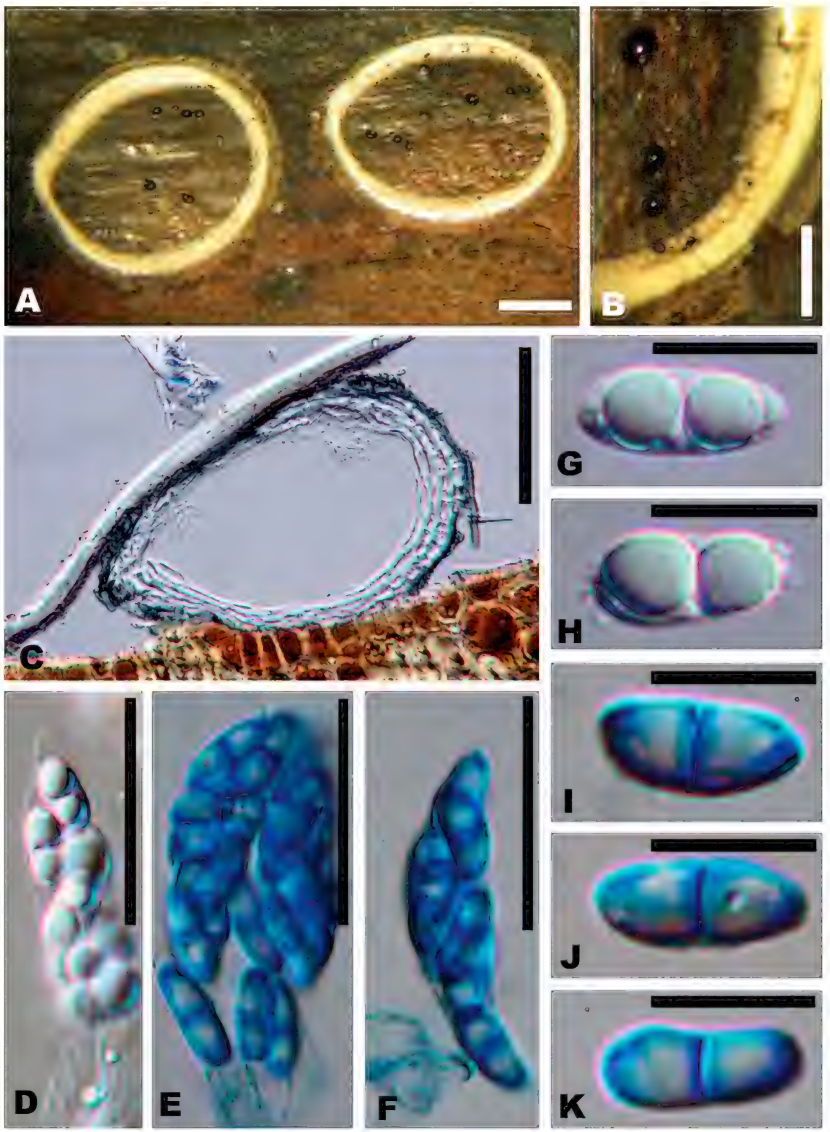

Plate 1. *Lignincola conchicola* (MFLU11 0032 holotype). A–B. Ascomata on host surface. C. Section of ascoma. D–F. Asci, G–K. Ascospores. C–D, G–H, in water, E–F, I–K, in cotton blue. Scale bars. A = 1000 µm, B = 500 µm, C = 50 µm, D–F = 30 µm, G–K = 10 µm.

absent. Asci 50–75 (–90) × 13–16 µm (Mean = 62 × 14 µm, n = 25), 8-spored, unitunicate, thin-walled, clavate to cylindrical-clavate, short stipitate, lacking

an apical apparatus and apical pore, persistent, arising from the base of ascoma. Ascospores 10.5–16 × 5–7 µm (Mean = 13 × 5.5 µm, n = 50), 1-septate, hyaline, ellipsoidal, frequently fusiform, with a slightly pale rose hue in transmitted light, appendages absent.

Habitat: Saprobic on adhesive disc of an invertebrate on submerged *Phoenix paludosa* fronds.

Distribution: Thailand.

Key to species of *Lignincola*:

1. Asci with an apical apparatus, ascospores cylindrical ..... ***L. nypae***
1. Asci without an apical pore, ascospores ellipsoidal 2
2. Ascospores thin-walled 3
2. Ascospores thick-walled, 22–36 × 12–16 µm ..... ***L. tropica***
3. Ascospores 13–24 × 5–8 µm ..... ***L. laevis***
3. Ascospores 10.5–16 × 5–7 µm; on the adhesive pad of a marine invertebrate ..... ***L. conchicola***

Discussion

Most taxa referred to *Halosphaeriaceae* are marine, usually with well-developed perithecioid ascomata that are globose, light or dark, and usually with an ostiole lined with periphyses (Müller & Arx 1962, Kohlmeyer 1972). Paraphyses are absent, catenophyses generally deliquesce if present, asci are clavate to ellipsoidal, thin-walled, unitunicate and deliquesce early, generally lacking an apical apparatus or pore, and ascospores are hyaline to light brown, unicellular or one to several septate, often with appendages or surrounded by a mucilaginous sheath (Shearer & Miller 1977, Jones 1995, Sakayaroj et al. 2011). A few transitional species of this family have been found in freshwater and brackish water habitats, including some species of *Aniptodera, Ascosacculus, Halosarpheia, Lignincola, Luttrellia* and *Nais* (Shearer & Miller 1977, Shearer 1989, Jones 1995, Pang et al. 2003a,b, Jones et al. 2009, Sakayaroj et al. 2011). The morphological characteristics of *Lignincola conchicola* fit well within this family.

Jones et al. (2009) suggested that the genus *Lignincola* has only one unifying character — the hyaline 1-septate ascospores without appendages. In *L. conchicola*, asci are persistent and thin-walled and lack an apical apparatus (Plate 1 D–F) and ascospores are thin-walled and lack appendages (Plate 1 G–K). *Lignincola conchicola* differs from other species in the genus in ascospore dimensions and the persistent occurrence of ascomata on the adhesive pad of a marine invertebrate.

Lignincola conchicola is most similar to *L. laevis*, the type species of the genus. The two taxa have similarly shaped asci and ascospores. They differ in that *L. laevis* has larger ascospores (13–24 × 5–8 µm) and the ascomata are

necked. *Lignincola tropica* differs from *L. conchicola* in having larger ascospores (22–36 × 12–16 µm) and in being thick-walled (Jones et al. 2009), *Lignincola nypae* can be distinguished from the new species by the presence of an apical apparatus in the ascus and cylindrical ascospores (Hyde et al. 1999, Pang et al. 2003a, Jones et al. 2009).

Three genera —*Aniptodera*, *Gesasha, Nais*— are also morphologically similar to *Lignincola* with some species with 1-septate, unappendaged ascospores (Shearer & Miller 1977, Kohlmeyer 1984, Crane & Shearer 1986, Abdel-Wahab & Nagahama 2011). In the type species of *Aniptodera*, the ascus is clavate with an apical pore and apical retraction of the plasmalemma, while the ascospores are thick-walled. Three species were described when Abdel-Wahab & Nagahama (2011) introduced *Gesasha*, and the ascospore dimensions and shape are quite similar to *L. conchicola*. However, *Gesasha* diagnostic characters include a periphysate neck, asci with an apical thickening, and pore features that exclude *L. conchicola* (Abdel-Wahab & Nagahama 2011). The two currently accepted *Nais* species differ from *L. conchicola* in ascospore dimensions and deliquescing asci (Pang et al. 2003a, Jones et al. 2009).

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Craspedodidymum and *Corynespora* spp. nov. and a new anamorph recorded from southern China

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Abstract — Two new species are described and illustrated: *Craspedodidymum fujianense* from dead branches of *Acacia confuse* and *Corynespora fujianensis* from dead branches of *Myrioneuron faberi*. *Craspedodidymum proliferans* is a new record for China. These three species were collected from tropical and subtropical forests in southern China.

Key words — microfungi, taxonomy

Introduction

China is considered an important world reservoir of biodiversity. Many wood-inhabiting fungi collected in China have recently been published (Dai et al. 2009, Dai & Li 2010, Ma et al. 2010). As part of studies on the fungal diversity of southern China, numerous collections of anamorphic fungi on dead wood were made from tropical and subtropical forests. Among these, two undescribed species and a new record were found. These three species are described, illustrated and compared with similar species. The specimens are deposited in HSAUP (Herbarium of the Department of Plant Pathology, Shandong Agricultural University) and HMAS (Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences).

Taxonomy

Craspedodidymum fujianense L.G. Ma & X.G. Zhang, **sp. nov.** Fig. 1

MycoBank MB 561700

Coloniae in substrato naturali effusae, atrobrunneae. Mycelium partim superficiale vel immersum. Conidiophora macronematosa, mononematosa, solitaria, simplicia, erecta, recta vel leniter flexuosa, laevia, crassitunicata, septata, brunnea vel atrobrunnea, ad apicem pallida, 240–320 μm longa, 6.5–8.5 μm lata. Cellulae conidiogenae integratae, terminales, monophialidicae, cylindricae, brunneae, 21.5–25 × 11–12 μm, cum collaretto cupulato, 6.5–7.5 μm longo, ad apicem 6.5–9.5 μm lato, ad basim 4.5–5.5 μm diamm

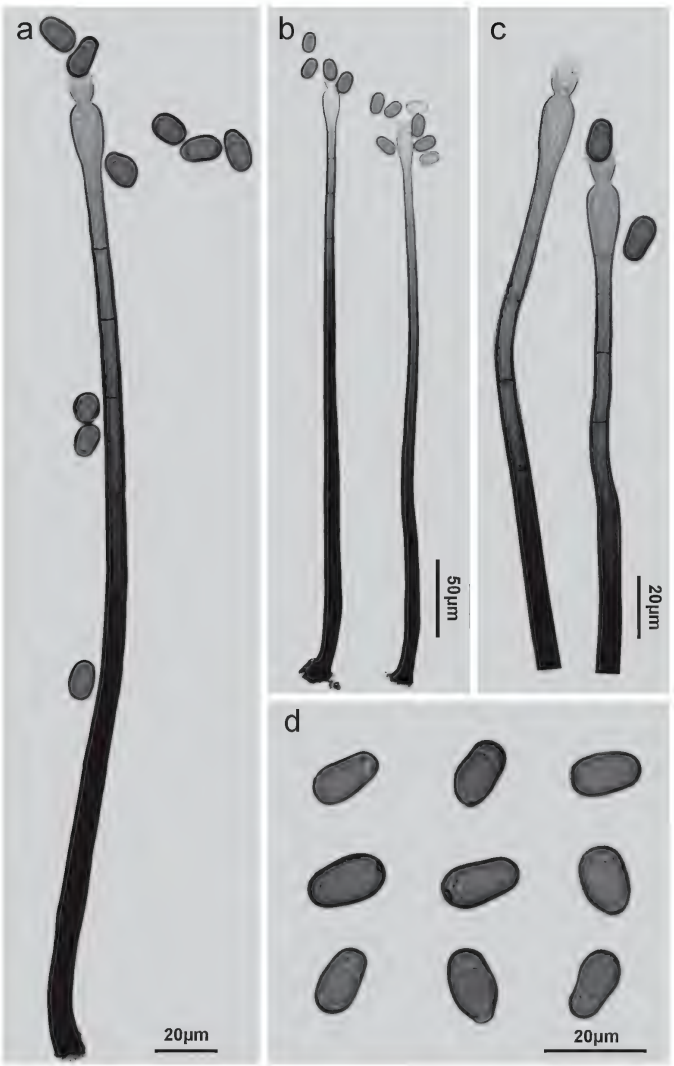

Fig. 1. *Craspedodidymum fujianense*. a, b. Conidiophores, conidiogenous cells and conidia. c. Conidiogenous cells with collarettes. d. Conidia.

praeditae. Conidia 13–15.5 × 7.5–10 μm, crassitunicata, laevia, brunnea, oblonga, 0-septata.

Type: China, Fujian Province: the National Forest Park of Wuyishan, on dead branches of *Acacia confusa* Merr. (*Mimosaceae*), 15 Aug. 2009, L.G. Ma (**holotype**, HSAUP H1010-2; **isotype** HMAS 146089).

Etymology: in reference to the province where the type was found.

Colonies on the natural substratum effuse, dark brown. Mycelium superficial and immersed. Conidiophores macronematous, mononematous, solitary, simple, erect, straight or slightly flexuous, smooth, thick-walled, septate, brown to dark brown, paler towards the apex, 240–320 µm long, 6.5–8.5 µm wide. Conidiogenous cells integrated, terminal, monophialidic, cylindrical, brown, swollen at the subapical region, 21.5–25 × 11–12 µm, with a collarette at the apex. Collarette funnel-shaped, 6.5–7.5 µm high, 6.5–9.5 µm wide at the apex, 4.5–5.5 µm diam. at the base. Conidia 13–15.5 × 7.5–10 µm, thick-walled, smooth, brown, oblong, 0-septate.

Holubová-Jechová (1972) established *Craspedodidymum* for *C. elatum* Hol.-Jech., the type species. The genus is characterized by macronematous, mononematous, simple or branched conidiophores bearing integrated, terminal, enteroblastic, monophialidic, cylindrical, apically swollen conidiogenous cells with a large and distinct funnel-shaped terminal collarette (Holubová-Jechová 1972, Ellis 1976, Yanna et al. 2000). *Craspedodidymum pulneyense* Subram. & Bhat has been placed in synonymy with *C. proliferans* (Bhat & Kendrick 1993). Eleven species are currently recognized in this genus, none of which were described from China. *Craspedodidymum* is reported for the first time from China.

Craspedodidymum fujianense is unique in this genus in producing oblong 0-septate conidia without a papilla. It most closely resembles *C. siamense* Pinruan, which also produces 0-septate conidia that are rounded at the base (Pinruan et al. 2004). However, *C. fujianense* differs by its oblong, shorter and wider conidia.

Craspedodidymum proliferans V. Rao & de Hoog, Stud. Mycol. 28: 64, 1986.

Fig. 2

Colonies on the natural substratum effuse, dark brown. Mycelium partly superficial and partly immersed. Conidiophores macronematous, mononematous, solitary, erect, simple, straight or slightly flexuous, percurrently proliferating, smooth, thick-walled, septate, brown to dark brown, unbranched, paler towards the apex, up to 350 µm long, 7.5–8.5 µm wide, slightly swollen at the base. Conidiogenous cells integrated, terminal, monophialidic, cylindrical, brown, swollen at the subapical region, 20.5–24 × 9.5–10 µm, with a collarette at the apex. Collarette funnel-shaped, 4.5–6.5 µm high, 7–7.5 µm diam. at the opening, narrowing to 4–4.5 µm diam at the base. Conidia 9.5–13 × 8.5–12 µm, thick-walled, smooth, dark brown, obovoid, subglobose to trapezoid, truncate at the base, 0-septate.

Specimen examined: China, Fujian Province: the National Forest Park of Wuyishan, on dead branches of *Magnolia paenetalauma* Dandy (*Magnoliaceae*), 15 Aug. 2009, L.G. Ma, HSAUP H1031 (duplicate HMAS 146090).

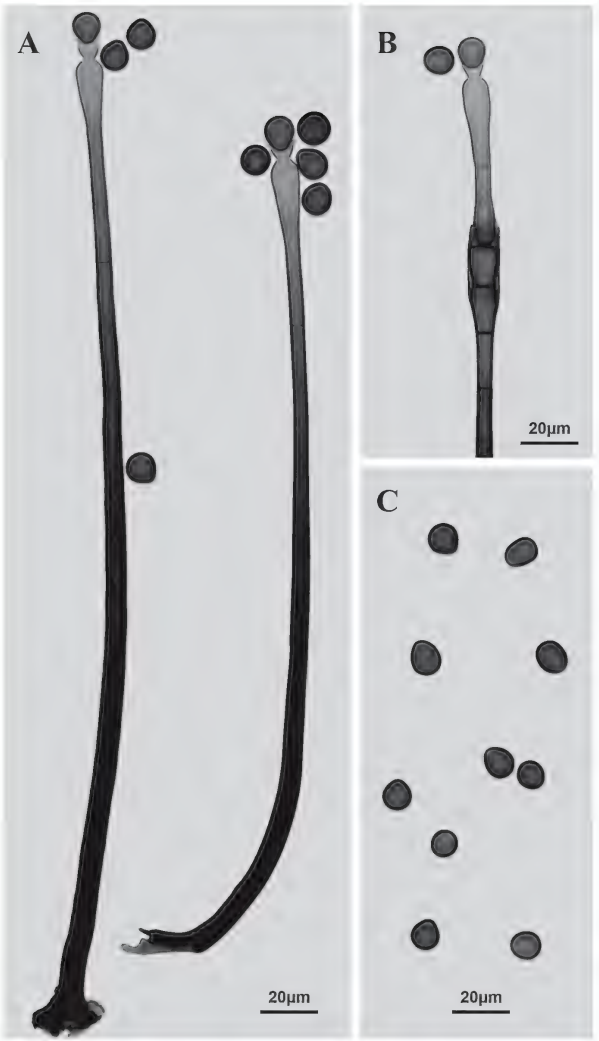

Fig. 2. *Craspedodidymum proliferans*. A. Conidiophores, conidiogenous cells and conidia. B. Conidiophore with percurrent proliferation. C. Conidia.

Craspedodidymum proliferans is reported for the first time from China. Compared with the morphological characters of the species as described by Rao & de Hoog (1986), both of the collections have obovoid, subglobose to trapezoid, 0-septate conidia with truncate base, with almost identical conidial size (9.5–13 × 8.5–12 μm vs. 10–14 × 8–11 μm). We believe they are the same

species. *C. proliferans* is most similar to *C. cubense* J. Mena & Mercado (Mercado & Mena 1992) in conidial shape, septation and base, but differs from *C. cubense* by its much narrower conidia.

Corynespora fujianensis L.G. Ma & X.G. Zhang, **sp. nov.** FIG. 3

MycoBank MB 561701

Coloniae in substrato naturali fuscae, effusae. Mycelium semper superficiale, ex hyphis ramosis, septatis, subhyalinis vel pallide brunneis, laevibus, 2–5 µm crassis compositum. Conidiophora singula vel fasciculata, erecta, interdum ramosa, recta, cylindrica, septata, verruculosa, pallide brunnea vel brunnea, per usque ad 7 proliferationes percurrentes successivas cylindricae elongascentia, 700–1300 µm longa, 4–5.5 µm crassa. Cellulae conidiogenae monotreticae, in conidiophoris incorporatae, crassitunicata, terminales, cylindricae, 9.5–14 µm longa, 6.5–9 µm crassa, brunnea. Conidia recta vel leviter curvata, singula, acrogena, obclavata, longer attenuata, laevia, brunnea, crassitunicata, 4–10-distoseptata, 31–90 × 6.5–10 µm.

Type: China, Fujian Province: the National Forest Park of Wuyishan, on dead branches of *Myrioneuron faberi* Hemsl. ex F.B. Forbes & Hemsl. (*Rubiaceae*), 15 Aug. 2009, L.G. Ma (**holotype**, HSAUP H1006-2; **isotype**, HMAS 146094).

Etymology: in reference to the province where the type was found.

Colonies on the natural substrate blackish brown, effuse. Mycelium mostly superficial, composed of branched, septate, subhyaline to pale brown, smooth-walled hyphae, 2–5 µm thick. Conidiophores arising singly or in groups, erect, sometimes branched, straight, cylindrical, septate, verruculose, pale brown to brown, with up to 7 successive percurrent cylindrical proliferations, 700–1300 µm long, 4–5.5 µm thick. Conidiogenous cells monotretic, integrated, terminal, thick-walled, cylindrical, 9.5–14 µm long, 6.5–9 µm wide, brown. Conidia straight or slightly curved, formed singly, acrogenous, obclavate, tapering to the apex, smooth, brown, thick-walled, becoming gradually paler towards the apex, 4–10-distoseptate, 31–90 × 6.5–10 µm.

The genus *Corynespora* was erected by Güssow (1906). Wei (1950) emended the diagnosis of the genus and clarified the conidiogenesis of *C. cassiicola* (Berk. & M.A. Curtis) C.T. Wei [= *C. mazei* Güssow, the type species]. *Corynespora* is characterized by macronematous, mononematous, simple, or branched conidiophores with monotretic, determinate, or percurrent conidiogenous cells, and obclavate to cylindrical, distoseptate, solitary, or catenate conidia. More than 100 species have been validly described under *Corynespora*, 24 of which were described from China, 4 parasitic on plant leaves (Guo 1984), and 20 saprobic on deciduous stems or wood (Zhang & Ji 2005, Zhang & Shi 2005, Zhang & Xu 2005, Shang & Zhang 2007, Wang & Zhang 2007, Zhang & Zhang 2007, Ma & Zhang 2007, Ma et al. 2008, Zhang et al. 2008, 2009).

Among described *Corynespora* species, *C. jasminicola* Meenu et al. (Meenu et al. 1998) and *C. combreti* M.B. Ellis (Ellis 1963) are most similar to *C. fujianensis*

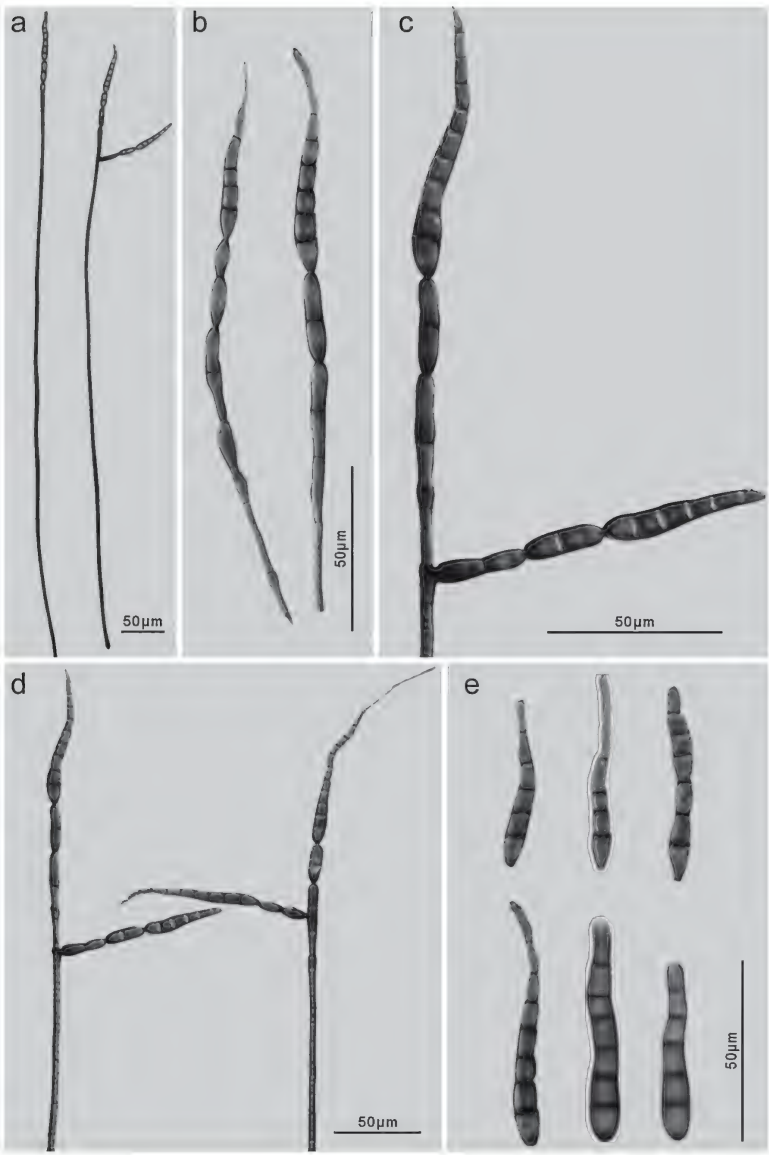

FIG. 3. *Corynespora fujianensis*. a. Conidiophores and conidia. b. Unbranched conidiophores and conidiogenous cells. c–d. Branched conidiophores and conidiogenous cells. e. Conidia.

in having obclavate conidia and branched conidiophores. The conidia of *C. fujianensis* are smaller with fewer septa than those of *C. jasminicola*, and

are not rostrate while those of *C. combreti* are sometimes rostrate. In addition, *C. fujianensis* differs by possessing verruculose and more slender conidiophores.

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A new species of *Lepiota* (*Agaricaceae*) from southwestern China

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Abstract — A new species, *Lepiota pallidiochracea*, is described. It is characterized by the ochraceous-red squamules on pileus, large ellipsoid to oblong spores, polymorphic cheilocystidia, and a trichodermium of elongate pileus covering, which is apically attenuate.

Key words — *Agaricales*, lepiotaceous fungi, taxonomy

Introduction

The eastern Himalaya is one of the twenty-five hotspots for biodiversity (Myers et al 2000), and macrofungi are very rich in the area (Yang 2005). Many new agarics and aphyllophoroid fungi have recently been described from there (Yang et al. 2004; Dai et al. 2007; Yuan & Dai 2008; Li et al. 2009; Dai 2010, 2011; Li et al. 2011). This paper is another contribution on the lepiotaceous fungi occurring in the region.

The genus *Lepiota* (Pers.) Gray contains more than 400 described species (Kirk et al 2008), and it is reasonable to expect many representatives of this cosmopolitan genus in China. However, rather few taxa have been originally described from China (Chiu 1948, Bi et al 1986, Yang 1994, Tolgor & Li 2004, Wang & Yang 2005a,b). In our research on *Lepiota* in China, in addition to several recently published new species (Liang et al 2009, 2010, 2011, Liang & Yang 2011), several interesting and undescribed taxa still await documentation. The present paper describes one new *Lepiota* species from eastern Himalaya.

Materials & methods

Macro-morphological features were recorded in the field. Material was dried using an electric drier and deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (HKAS) and Research Institute of Tropical

Forestry, Chinese Academy of Forestry (RITF). Terminology for descriptive terms follows Vellinga & Noordeloos (2001). Color designations are from Kornerup and Wanscher (1981).

For microscopic observations, sections of fruitbodies were made by hand and mounted in 5% KOH, Congo red, and Melzer's reagent. Basidiospores were mounted in cresyl blue to test for a metachromatic reaction (Singer 1986). Size ranges were determined for basidia, basidiospores, cheilocystidia, and elements of the pileipellis, based on ocular micrometer measurements of at least 20 elements of each character. The abbreviation [n/m/p] indicates that measurements were made on n basidiospores in m basidiomata from p collections. Dimensions of basidiospores are given using a notation of the form (a–)b–c(–d). The range b–c contains a minimum of 90% of the measured values. Extreme values are given in parentheses. The following abbreviations are used: Q refers to the length/breadth ratio of basidiospores; **Q** refers to the average Q of all basidiospores ± sample standard deviation.

Taxonomy

Lepiota pallidiochracea J.F. Liang & Zhu L. Yang, **sp. nov.** Fig. 1–4

Mycobank 561241

Pileus sordide albus, squamulis densibus, rubro-ochraceis, rubro-brunneis vel brunneis. Stipes subcylindricus, squamulis brunneis. Basidiosporae 9.0–12.0 × 6.0–9.0 µm, ellipsoideae vel lato fusiformes. Cheilocystidia diversa, clavata, fusiformia vel hamata. Pleurocystidia nulla. Squamulae pilei ex trichodermiis attenuatis terminalibus compositae. Fibulae praesentes.

Type: China, Tibet, Changdu Co., near Qiongka Bridge, 27 July 2004, Zhu L. Yang 4194 (HKAS 45579 **holotype**).

Etymology: *pallidiochracea* (Lat.), referring to pale ochraceous squamules on the pileus.

Basidiomata (Fig. 1) small-sized. Pileus 1.5–4 cm diam., campanulate with small umbo when young, plano-convex with obtuse umbo at maturity, cream to whitish, at centre brownish red (10D6-8) to dark brown (9E4-8), around centre densely covered with concentric squamules; squamules more or less uplifted, minute, ochraceous-red (8C7-8), brown (7D8) to reddish brown (9C7-8). Lamellae free, subventricose, moderately crowded with lamellulae, dirty white to cream. Stipe 2–4 × 0.2–0.3 cm, subcylindrical, hollow; surface glabrous and white at the apex, light ochraceous (7C6-7) with scattered ochraceous-red (8C7-8), brown (7D8) to reddish brown (9C7-8) squamules at the low part. Annulus whitish, membranous, evanescent. Odor none. Taste not recorded.

Basidiospores (Fig. 2) [63/3/2] 9–12(–14) × 6–8(–9) µm [Q = 1.29–1.85, **Q** = 1.56 ± 0.18], ellipsoid to oblong, sometimes ovoid with small hilar appendage in side view, with neither suprahilar depression nor germ pore, ellipsoid in frontal view; hyaline, smooth, slightly thick-walled, dextrinoid, weakly congophilic, not metachromatic in Cresyl Blue. Basidia 25–37 × 9–14 µm, clavate, 4-spored, rarely 2-spored. Lamella edge sterile. Cheilocystidia

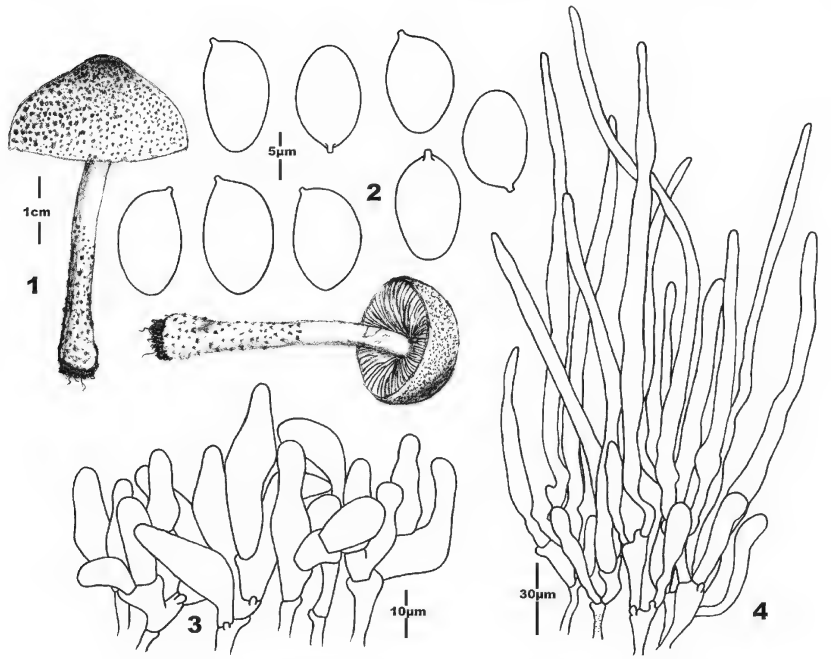

Fig. 1–4 *Lepiota pallidiochracea* (All from holotype, HKAS 45579).
1. Basidiomata. 2. Basidiospores. 3. Cheilocystidia. 4. Pileus covering.

(Fig. 3) 15–40 × 5–10 μm, variable in shape, subcylindrical to narrowly clavate, or fusiform, lageniform to utriform; walls smooth, thin, hyaline in KOH, congophilic. Pleurocystidia absent. Pileus covering (Fig. 4) a trichodermium of elongate, apically attenuate terminal elements 60–256 × 6.5–11 μm, often with some short narrowly clavate elements interspersed, with pale yellowish brown parietal and intracellular pigment in upper part; fine incrustation sometimes in basal part of elements. Clamp connections common.

Additional specimen examined: **China: Yunnan Prov.**, Shangri-La County, Daxiagu, alt. 3060 m, 20 July 2008, Jun F. Liang 799 (RITF 660).

Habitat & distribution: in small groups, gregarious, saprotrophic and terrestrial on slope, in summer. Known only from southwestern China.

Discussion: *Lepiota pallidiochracea* is characterized by its ochraceous-red squamules on the pileus, which is covered with a trichodermium of long, elongate, apically attenuate and short narrowly clavate elements, large ellipsoid to oblong spores, and polymorphic cheilocystidia.

Based on the trichodermium type of the pileus squamules and the ellipsoid basidiospores, *L. pallidiochracea* may tentatively be placed in *L.* sect. *Ovisporae*

(J.E. Lange) Kühner (Singer 1986, Vellinga 2001), although species in the section usually have spores smaller than 10 μm (Singer 1986). Recent molecular phylogenetic studies indicate that section *Ovisporae* is not monophyletic, so that re-evaluation of this section is needed (Vellinga 2003, Liang et al 2011). Phylogenetically, the new species may be referred to section *Lepiota* or clade 1 of *Lepiota* s.l. (Vellinga 2003) until a new taxonomic system is proposed.

Several *Lepiota* species have similarly shaped spores. The polymorphic cheilocystidia coupled with the larger spores help distinguish *L. pallidiochracea* from *L. brunneolilacea* Bon & Boiffard (also a member of *L.* sect. *Ovisporae*), *L. helveola* Bres. and *L. ochraceoaurantiaca* Dennis (Bon 1996, Candusso & Lanzoni 1990, Dennis 1952). The larger spores are reminiscent of *L. oreadiformis* Velen. (in sect. *Lepiota*), which, however, has much larger, fusiform to amygdaliform spores and narrowly clavate or utriform cheilocystidia (Vellinga 2001).

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MYCOTAXON

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Passalora wangii comb. nov. from the genus *Tandonella*

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Abstract —The anamorphic fungus *Tandonella wangii* is recombined as *Passalora wangii*. The species was originally collected on leaves of *Ligularia* sp. during a taxonomic survey carried out in Motianling, Inner Mongolia, China.

Key words — hyphomycete, imperfect fungi, taxonomy

Tandonella S.S. Prasad & R.A.B. Verma (Prasad & Verma 1970) was one of the anamorph genera for *Mycosphaerella*, usually plant pathogenic, symptomless or almost so but also often causing leaf lesions. Differentiating between *Passalora* and *Tandonella*, which comprised taxa with superficial secondary mycelium, synnematous conidiophores, and catenate conidia, was difficult.

When Crous & Braun (2003: 19–22) emended the circumscription of *Passalora*, they reduced *Tandonella* to a synonym of *Passalora*, which otherwise differed from *Tandonella* by the formation of solitary conidia and the absence of superficial mycelium and synnematous conidiophores. The emended *Passalora* embraces a wide morphological variation with secondary mycelium that is absent or well developed, external, and superficial, conidiophores that are solitary, fasciculate, or in sporodochial to synnematous conidiomata, and conidia that range from solitary to catenate and in simple or branched chains.

Because *Tandonella* is no longer tenable as a separate genus, we here transfer the previously reported species, *T. wangii* (Zhai et al. 2006), to *Passalora*.

Passalora wangii (F.Y. Zhai, Y.L. Guo & Yu Li) F.Y. Zhai, Y.L. Guo & Yu Li, **comb. nov.**

MycoBank: MB519619

≡ *Tandonella wangii* F.Y. Zhai, Y.L. Guo & Yu Li, Mycosystema 25(3): 374. 2006.

Description and illustration: Zhai et al (2006: 375, Fig.1).

Habitat and distribution: Known only from the type collection, on leaves of *Ligularia* sp. (*Asteraceae*), from Inner Mongolia.

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A new species of *Terriera* (*Rhytismatales*, *Ascomycota*) from China

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Abstract —A new *Terriera* species, *T. huangshanensis* on leaves of *Eurya muricata* var. *huiana*, is described. The species is placed in *Terriera* based on the presence of strongly carbonized extensions adjacent to the ascoma opening, somewhat thin-walled cells at the marginal parts of the ascoma composed of colorless to light brown textura angularis-prismatica, and the absence of lip cells. The new species is similar to *T. minor*, which is distinguished by smaller ascomata with rounded ends, textura prismatica in the corner between the covering and basal stroma, paraphyses branching 2–3 times in the apical 30–40µm, sequentially ripening asci, and ascospores tapering towards the ends. The type specimen is deposited in the Herbarium of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words —taxonomy, *Rhytismataceae*, *Theaceae*

Introduction

Terriera B. Erikss. is a member of *Rhytismataceae* (*Rhytismatales*, *Leotiomycetes*, *Ascomycota*) (Kirk et al. 2008). Since Eriksson (1970) established *Terriera* as a new genus for *T. cladophila* (Lév.) B. Erikss., 17 species and 2 varieties have been reported. *Terriera* species are distributed worldwide and associate with both angiosperms and gymnosperms. In the past, members of this genus were placed in *Lophodermium* Chevall., *Hypoderma* De Not., *Clithris* (Fr.) Bonord., and *Dermascia* Tehon (Johnston, 2001). Although the classifications of Höhnel (1917) and Tehon (1935), based heavily on developmental characteristics of the ascomatal primordium, have been criticized and rejected by later researchers, they appear highly significant for recognizing *Terriera* as phylogenetically distinct from the highly heterogeneous *Lophodermium* s. lat. (Lantz et al. 2011). Ortiz-García et al. (2003) noted that '*Terriera minor* differs from *Lophodermium* in structure of the ascomatal primordium.' Johnston (2001) emphasized the importance of characters such as ascus and ascospore size, apical shape of the ascus and paraphysis, and features of the conidiomata

in his taxonomy of *Terriera*. Therefore in Johnston (2001) and Ortiz-García et al. (2003), he accepted into the genus several species with a typically *Terriera*-like ascoma such as *Clithris arundinacea* Penz. & Sacc., *Hypoderma fuegianum* (Speg.) Kuntze, *Lophodermium javanicum* Penz. & Sacc., and *L. sacchari* Lyon. Ortiz-García et al. (2003) attempted to explain phylogenetic relationships within *Lophodermium* by rDNA-ITS sequence analyses of *Lophodermium* species and representatives of other rhytismataceous genera. The results showed that *Lophodermium* from pine hosts formed an independent clade with *Meloderma* Darker and *Elytroderma* Darker but were distally related to *Terriera*. A more comprehensive survey by Lantz et al. (2011) indicated that *Terriera* is monophyletic, while *Lophodermium* as currently circumscribed contains as many as 10 independently evolved species clusters.

For China, only one species of *Terriera*, *T. brevis* (Berk.) P.R. Johnst., was reported in Hong Kong (Fröhlich & Hyde 2000). In the present paper we describe a new *Terriera* species from the Huangshan Mountains in Anhui Province, China.

Materials & methods

Mature fruit bodies were selected from the collected specimen. External shapes, size, color and opening ways of ascomata and conidiomata as well as zone line were observed under the dissecting microscope. Materials were then rehydrated in water for 15min, after which 10–15 μm thick fruitbody sections were cut by a freezing microtome and mounted in lactic acid or cotton blue with water pretreatment for viewing outlines of ascomata and conidiomata in vertical section. Gelatinous sheaths surrounding ascospores and paraphyses were examined in water or 0.1% (w/v) cotton blue in lactic acid. Color of diversified structures and ascospore contents were observed in water. Measurements and drawings were made using materials mounted in 5% KOH and from 30 asci, ascospores, and paraphyses for each specimen. Line and point integrated illustrations of internal structures of fruit bodies were drawn using the microscopic painting device (Panasoianic XSJ-2). The type specimen is deposited in the Herbarium of Forest Fungi of Anhui Agricultural University, China (AAUF).

Taxonomy

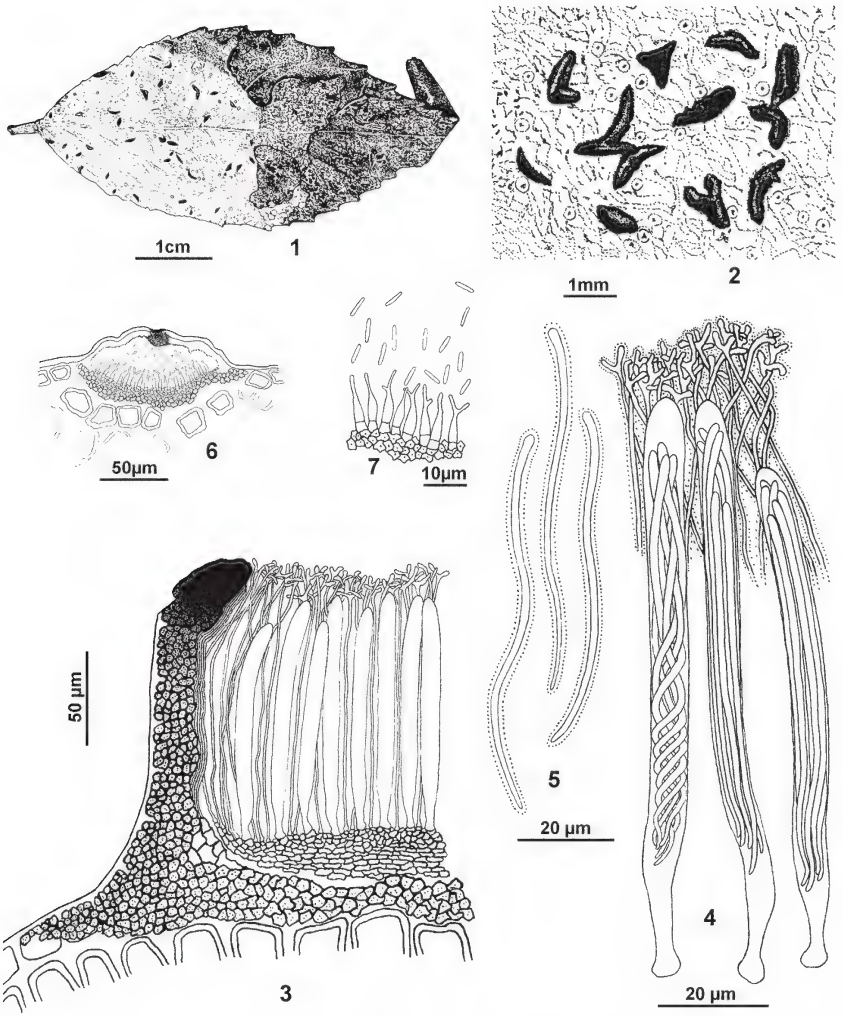
Terriera huangshanensis Z.Z. Yang, Y.R. Lin **& C.L. Hou, sp. nov.** FIGURES 1–7

MYCOBANK 512114

Ascomata amplitudine admodum variabilia, 550–1900 × 200–550 μm, elliptica, subfusiformia vel rimis ternis aperientia. Paraphyses filiformes, ad apicem plerumque gradatim tumidae vel irregulatim semel aut iterum ramosae. Asci in simul maturescentes, 8-spori. Ascosporae 58–90 × 1.5–2 μm, filiformes, hyalinae, aseptatae, vagina gelatinosa 1–1.5 μm crassa indutae.

TYPE: On leaves of *Eurya muricata* var. *huiana* (Kobuski) Hu & L.K. Ling (Theaceae), China. Anhui, Mt Huangshan, Wenquan. alt. ca 700 m, 12 June 2006, Y. R. Lin et al. L2217 (**HOLOTYPE** AAUF68325).

ETYMOLOGY: *huangshanensis*, referring to the place where the specimen was collected.



Figs 1–7. *Terriera huangshanensis* on fallen leaves of *Eurya muricata* var. *huiana*. 1. Habit on leaf. 2. Detail of ascomata and conidiomata. 3. Portion of ascoma in median vertical section. 4. Paraphyses and asci. 5. Discharged ascospores. 6. Conidioma in vertical section. 7. Conidiogenous cells and conidia.

Ascomata developing on both sides of fallen leaves, principally on the upper side of the leaf, scattered to clustered, sometimes confluent in groups of two or three, in drab or gray-white bleached areas 17–25 mm diam. In surface view, ascomata varied in dimensions, 550–1900 ×200–550 µm, elliptical, fusiform

or subfusiform, straight or curved (lunate), sometimes 3-lobed or triangular, ends rounded to subacute, strongly raised above the surface of the substrate at maturity, opening by a single longitudinal split which is branched in the triangular ascomata. Lips absent, split extending almost the whole length of the ascoma. Entirety of ascomata black, matt or slightly glossy, the edge well defined. In median vertical section, ascomata subepidermal with epidermal cells becoming filled with fungal tissue as ascomata develops, 175–205 µm deep. Covering stroma 18–22 µm thick near the centre of the ascoma, slightly thicker towards the edges, extending to the basal stroma, consisting of dark brown to light black, thick-walled textura angularis and textura globulosa with cells 3.5–5.5 µm diam. Along the edge of the ascoma opening there is a 12–20 µm thick extension adjacent to the covering stroma which covers the hymenium, and which is comprised of strongly carbonized tissue with no obvious cellular structure. Excipulum very poorly developed, closely adhering to sides of the extension and the covering stroma. Basal stroma 10–18 µm thick, dark brown, consisting of 2–4 rows of 4–7 µm diam., angular, thick-walled cells. Subhymenium 15–25 µm thick, composed of hyaline textura angularis and textura porrecta, with a colorless to gray-brown, 12 –30 µm thick, of textura angularis mixed with textura prismatica at the edge of the ascoma. Hymenium often extending beyond the top of the extension when ripening. Paraphyses 120–140 × 1–1.2 µm, filiform, thin-walled, hyaline, branching 1–2 times and slightly swollen at the apex, with a ca 1 µm thick gelatinous matrix, forming a distinct epithecium above the asci. Asci ripening synchronously, 100–120 × 5–7 µm, narrow-cylindrical, thin-walled and equal, apex rounded or subacute, not bluing in iodine, discharging spores through a small apical hole, 8-spored, stalk 15–28 µm in length. Ascospores borne in a fascicle, sometimes helically arranged, 58–90 × 1.5–2 µm, filiform, slightly tapered towards the base, hyaline, aseptate, thin-walled, covered by a 1–1.5 µm thick gelatinous sheath.

Conidiomata on both sides of leaves, predominantly on the upper side, scattered to crowded, sometimes coalescing. In surface view, conidiomata 75–130 × 70–90 µm, round to elliptical, raising the leaf surface, lightly brown but dark brown, grey-brown or dark brown in the regions of the edge and the surrounding of apical ostiole after discharging spores. In vertical section, conidiomata subcuticular, somewhat lenticular in outline, 50–65 µm deep. Upper wall poorly developed, only present in surrounding of the ostiole. Basal wall well-developed, 6–8.5 µm, dark brown, consisting of 2–3 rows of 2–3.5 µm diam., angular, slightly thick-walled cells. Subconidiogenous layer ca 8 µm thick, composed of very light, thin-walled, angular cells. Conidiogenous cells 8–12 × 2–3 µm, cylindrical, slightly tapered towards the apex, hyaline, proliferating sympodially. Conidia 4.5–6 × ca 1 µm, cylindrical, hyaline, aseptate.

Comments —*Terriera huangshanensis* is distinctive within the genus because of the synchronously ripening asci and the hymenium that often overtops the extension of the covering stroma when mature. The most widely distributed species, *T. minor* (Tehon) P.R. Johnst., is similar but differs in many aspects: its ascomata have rounded ends and are not associated with conidiomata and zone lines, the textura prismatica between the covering and basal stroma is poorly developed, asci ripen sequentially, and the ascospores are 0–1 septate and taper slightly towards both ends (Johnston 1988, 1989a, b). The type species *T. cladophila* is distinguished from *T. huangshanensis* by subcuticular, circular to elliptical, non-curved ascomata that are associated with brown diffuse zone lines; the textura prismatica between the covering and basal stroma composed of vertically oriented cells; sequentially ripening asci; and a subhymenium consisting of textura angularis and textura intricata (Minter 1996).

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New Bulgarian records of fungi associated with glacial relict plants

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Abstract — The paper contributes to knowledge of Bulgarian fungal diversity on glacial relict vascular plants. Eleven new country records of ascomycetes and anamorphic fungi are presented with descriptions, namely *Asteromella silvarum, Cainiella johansonii, Gnomoniella vagans, Hendersonia culmicola, Leptosphaerulina dryadis, Melasmia mougeotii, Phaeosphaeria nardi, Pseudomassaria islandica, Septoria macropoda, S. tenella,* and *Sydowiella dryadis. Microbotryum silenes-acaulis* is also an addition to the list reflecting the recent changes in *M. violaceum* s. lat. New data are included about the distribution of *Leptosphaeria doliolum, Nodulosphaeria modesta, Ophiognomonia gei-montani,* and *Phaeosphaeria juncina,* all previously recorded in Bulgaria. In addition, a brief review is presented of previously encountered fungal species on glacial relicts in the country.

Key words — taxonomy

Introduction

The study of fungi on relict hosts is of considerable interest as it may contribute to understanding fungal biogeography and fungus-host interactions, given that many parasitic and saprotrophic fungi demonstrate preferences for particular host plants. This research also contributes to better understanding of the need to establish appropriate practices for simultaneous conservation of specialized parasitic and saprotrophic fungi and their hosts (Denchev & Bakalova 2002, Denchev 2005). Research into fungal diversity associated with glacial relict plants has a long history. See Chlebicki (2002) for details and further references on this topic.

Although fungi associated with glacial relict plants have not previously been studied in Bulgaria, earlier taxonomic surveys have revealed a number of ascomycetes, smut, rust, and anamorphic fungi on various Bulgarian relict plants: on *Astragalus alopecurus: Uromyces punctatus* J. Schröt.; on *Carex atrata:*

Puccinia dioicae Magnus; on *Carex curvula: Anthracoidea curvulae* Vánky & Kukkonen and *Schizonella melanogramma* (DC.) J. Schröt.; on *Carex rupestris: Comoclathris deflectens* (P. Karst.) Nograsek and *Physalospora alpestris* Niessl; on *Cerastium eriophorum: Massariosphaeria rubicunda* (Niessl) Crivelli; on *Dianthus microlepis: Microbotryum dianthorum* (Liro) H. Scholz & I. Scholz; on *Dryas octopetala* s. lat.: *Isothea rhytismoides* (Bab. ex Berk.) Fr. and *Wettsteinina dryadis* (Rostr.) Petr.; on *Empetrum nigrum: Duplicaria empetri* (Fr.) Fuckel; on *Gentiana asclepiadea: Cronartium flaccidum* (Alb. & Schwein.) G. Winter; on *Oxyria digyna: Bauhinus vinosus* (Tul. & C. Tul.) R.T. Moore (≡ *Microbotryum vinosum* (Tul. & C. Tul.) Denchev) and *Puccinia oxyriae* Fuckel; on *Poa alpina: Puccinia graminis* Pers.; on *Polygonum viviparum: Bauhinus bistortarum* (DC.) Denchev (≡ *Microbotryum bistortarum* (DC.) Vánky); on *Primula deorum: Uromyces primulae-integrifoliae* (DC.) Niessl; on *Primula minima: Uromyces apiosporus* Hazsl.; on *Rheum rhaponticum: Oidium erysiphoides* Fr.; on *Salix herbacea: Rhytisma salicinum* (Pers.) Fr. and *Melampsora epitea* Thüm.; on *Salix lapponum: Hyaloscypha albohyalina* (P. Karst.) Boud. and *Melampsora lapponum* Lindf.; on *Salix reticulata: Melampsora epitea;* on *Saxifraga stellaris: Puccinia saxifragae* Schltdl.; on *Vaccinium uliginosum: Naohidemyces vacciniorum* (J. Schröt.) Spooner (Hinkova 1960, 1961; Fakirova 1991; Denchev 1994, 1995, 2001; Dimitrova 1995; Denchev & Negrean 2001; Chlebicki 2002; Zwetko et al. 2004).

The glacial refugia in Bulgaria are of undoubted interest, being among the southernmost points in Europe for distribution of a significant number of relict host plants. The authors of this paper aim at further detailed study of the mycota associated with glacial relict plants in Bulgaria. This initial study has focused on some parasitic and saprotrophic fungi on relict vascular plants less sampled during previous mycological studies in this country. The survey has so far produced some interesting new records that are reported below.

Material & methods

Fungi on *Antennaria dioica* (L.) Gaertn. (*Asteraceae*), *Bartsia alpina* L. (*Scrophulariaceae*), *Carex atrata* L. (*Cyperaceae*), *Dryas octopetala* L. (*Rosaceae*), *Festuca pirinica* Horvat ex Markgr.-Dann. (*Poaceae*), *Geum bulgaricum* Pančić (*Rosaceae*), *Juncus trifidus* L. (*Juncaceae*), *Kobresia myosuroides* (Vill.) Fiori (*Cyperaceae*), *Poa alpina* L. (*Poaceae*), and *Silene acaulis* (L.) Jacq. (*Caryophyllaceae*) were examined. Specimens were collected from three major Bulgarian glacial refugia — Vihren area (Pirin Mts), Kozyata Stena area (Stara Planina Mts), and the high mountain zone of Rila Mts (Fig. 1). These were supplemented by examination of older collections of glacial relict plants kept in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (SOM). All fungal specimens studied have been conserved in the Mycological Collection of the Institute of Biodiversity and Ecosystem Research (SOMF).

Fig. 1. Collecting areas for fungi on glacial relicts:
1. Kozyata Stena area (Stara Planina Mts), 2. Rila Mts, 3. Vihren area (Pirin Mts).

Microscopic examination was carried out on dried specimens. Slides of ascomata and conidiomata for LM were prepared in lactophenol, the mounting medium being gently heated to boiling point and then cooled. The descriptions below are based on the examined specimens. Primary sources employed for identification of fungi were the works of Holm (1952, 1979), Müller (1957), Barr (1965), Saville (1968), Arx (1970), Vassiljeva (1979, 1987, 1998), Merezhko (1980), Monod (1983), Vanev & van der Aa (1998), and Chlebicki (2002).

Ascomycetes new for Bulgaria

Cainiella johansonii (Rehm) E. Müll., Sydowia 10: 121, 1957['1956'].

Perithecia 100–115 × 170–205 µm, scattered, immersed, with a protruding beak. Beak 650 × 60 µm, long. Asci 137–141 × 37–41 µm, oblong, almost sessile, with refractive apical ring, I–, 8-spored. Ascospores (25–)27.0±1.9 (–32) × (11.5–)13.5±1.6(–19) µm, l/w (1.5–)2.02±0.17(–2.2) (n = 50), almost kidney-shaped, hyaline, finally brown, 1-septate (sometimes constricted at the septum).

Specimens examined — On petioles of *Dryas octopetala:* Stara Planina Mts, Kozyata Stena reserve, 17 Aug 2009, D.Y. Stoykov, B. Assyov & C.M. Denchev (SOMF 29 178); ditto, near the Boba peak, 42°47′05.5″ N, 24°32′45.9″ E, 18 Aug 2009, D.Y. Stoykov, B. Assyov & C.M. Denchev (SOMF 29 173).

Comments — *Cainiella johansonii* was described from Germany (Rehm 1904, as *Lizonia johansonii*) and further reported by Müller (1957) from the Alps (Italy) on *Dryas octopetala*. Later it was recorded in Canadian Arctic on *D. drummondii* Richardson ex Hook. and *D. integrifolia* Vahl (Chlebicki 2002). According to Holm (1979), this fungus is rather common in the Scandes Mountains (Scandinavia).

Gnomoniella vagans Johanson, Öfvers. Kongl. Svenska Vetensk.-Akad. Förh. 41(9): 163, 1884.

Perithecia densely scattered, immersed in leaf tissues. Neck long, protruding. Asci 45–70 × 10–16 µm, clavate, with apical refractive ring, I^-, 8-spored. Ascospores (13.5–)16.2±1.3(–18.5) × (5–)7.2±0.9(–8.5) µm (n = 25), ellipsoid to ovoid, hyaline, non septate.

Specimen examined — On dead leaves of *Dryas octopetala:* Stara Planina Mts, Ushite peak, 13 May 2002, leg. B. Assyov, det. D.Y. Stoykov (SOMF 29 172).

Comments — Barr (1959) considers this fungus to be a rare species.

Leptosphaerulina dryadis (Starbäck) L. Holm, Bot. Not. 132: 86, 1979.

Perithecia small, subpyriform, immersed in leaf tissues. Asci 70–81 × 30–33 µm, very few, saccate, 8-spored. Ascospores 27–34 × 10–12 µm, hyaline, about slipper shaped, upper part rounded, cylindrical below, with 5 transverse septa and one ± incomplete longitudinal septum; several additional transverse and longitudinal septa formed at maturity.

Specimen examined — On dead leaves of *Dryas octopetala:* Stara Planina Mts, Kozyata Stena reserve, near the Boba peak, 42°47′05.5″ N, 24°32′45.9″ E, 18 Aug 2009, D.Y. Stoykov, B. Assyov & C.M. Denchev (SOMF 29 174).

Comments — According to Holm (1979), this fungus is rare. The author suggested that this species is closely related to *Leptosphaerulina pulchra* (G. Winter) M.E. Barr on *Potentilla* spp., the latter distinguished on the basis of less pronounced ascospore septation than in *L. dryadis*.

Phaeosphaeria nardi (Fr.) L. Holm, Symb. Bot. Upsal. 14(3): 124, 1957.

Perithecia single, subepidermal, globose or depressed globose. Asci 62–82 × 9–13 µm, cylindric, 8-spored. Ascospores (19.5–)21.9±3.1(–27.5) × (3–)4.5±0.7(–5.5) µm (n = 50), yellowish to brownish, 7–8-septate; the third (fourth) cell from above swollen, of the same length as the lowest cells and distinctly longer than the second (third) cell; biseriate in the ascus.

Specimen examined — On dry leaves of *Kobresia myosuroides:* Pirin Mts, Kazana, on marble, 1 Oct 1999, leg. P. Vasilev, det. D.Y. Stoykov (SOMF 27 967).

Pseudomassaria islandica (Johanson) M.E. Barr, Mycologia 56: 854, 1965 ['1964'].

Perithecia 190–260 µm in diameter, subglobose, immersed. Setae 200–350 × 17.5–18 µm, dark-brownish. Asci 80–105 × 17.5–22 µm, ±oblong, with

refractive apical ring, I+, 8-spored. Ascospores (21.5–)23.0±1.6(–26.5) × (10.5–)10.7±0.8(–12.5) µm (n = 75), ellipsoid, yellowish-hyaline, apiosporous; irregularly uniseriate in the ascus.

Specimens examined — On dead leaves of *Dryas octopetala:* Pirin Mts, along the track from Vihren peak to Kazana, 2400 m, 41°76′88.7″ N, 23°41′25.2″ E, 11 Aug 2010, leg. I. Apostolova & H. Predashenko, det. D.Y. Stoykov (SOMF 27 631); Rila Mts, Sedemte Ezera, above Babreka lake, 2380 m, 42°12′07.6″ N, 23°18′27.2″ E, 15 Sep 2009, leg. I. Apostolova, det. D.Y. Stoykov (SOMF 27 640).

Sydowiella dryadis Lar.N. Vassiljeva, Mikol. Fitopatol. 13(4): 279, 1979.

Perithecia ca. 400 µm in diameter, superficial, single or in groups, black. Beak 350–700 × 100–120 µm, papillate. Asci 135 × 13 µm, cylindric, 8-spored, with apical ring. Ascospores (14–)16.9±1.7(–19.5) × (6–)7.4±0.62(–9) µm, l/w (1.9–)2.3±0.26(–2.9) (n = 75), ellipsoid, hyaline, two-celled, septum median, slightly constricted at the septa; uniseriate in the ascus.

Specimen examined — On dead twigs of *Dryas octopetala:* Stara Planina Mts, Kozyata Stena reserve, 17 Aug 2009, D.Y. Stoykov, B. Assyov & C.M. Denchev (SOMF 29 177).

Comments — The measurements of asci and ascospores of the Bulgarian specimen fit well those in the original description of the species (Vassiljeva 1979).

Ascomycetes already known from Bulgaria, found on glacial relict plants during the study

Leptosphaeria doliolum (Pers.) Ces. & De Not., Comment. Soc. Crittog. Ital. 1(4): 234, 1863.

Specimen examined — On dry leaves of *Poa alpina:* Stara Planina Mts, Sveti Plast peak, 1820 m, 16 Jul 1952 (ex SOM), fungus comm. & det. D.Y. Stoykov (SOMF 29 175).

Nodulosphaeria modesta (Desm.) Munk ex L. Holm, Symb. Bot. Upsal. 14(3): 80, 1957.

Specimen examined — On dead stems and floral parts of *Antennaria dioica:* Rila Mts, Sedemte Ezera, 7 Jul 2010, D.Y. Stoykov & C.M. Denchev (SOMF 27 629).

Comments — The fungus is so far known in Bulgaria only from Rila Mts on stems and leaves of *Lactuca* (Stoykov 2004).

Ophiognomonia gei-montani (Ranoj.) Sogonov, Stud. Mycol. 62: 58, 2008.

Specimens examined — On dead leaves of *Geum bulgaricum:* Rila Mts, Sedemte Ezera, ca. 2300 m, 21 Jul 1909, leg. B. Davidov (ex SOM), fungus comm. & det. D.Y. Stoykov (SOMF 29 171); Rila Mts, along the track from Smrudlivoto lake to Kirilova Polyana locality, 21 Jul 1998, D.Y. Stoykov (SOMF 22 511); Rila Mts, Yakoroudski circus, on the slopes of Mt Kovatch, above Murtvoto Ezero, ca. 2200 m, 30 Aug 2008, leg. B. Assyov & R. Vassilev, det. D.Y. Stoykov (SOMF 29 179).

Comments — A description of this species based on Bulgarian specimens on *Geum bulgaricum* appears in Stoikov (2000).

Phaeosphaeria juncina (Auersw.) L. Holm, Symb. Bot. Upsal. 14(3): 127, 1957.

Specimen examined — On leaves and floral parts of *Juncus trifidus:* Rila Mts, Sedemte Ezera, in a rock crack, 42°12′97.2″ N, 23°19′21.0″ E, 7 Jul 2010, D.Y. Stoykov & C.M. Denchev (SOMF 27 625).

Comments — In Bulgaria so far recorded only from Vitosha Mt, on *Juncus conglomeratus* L.

Anamorphic fungi new for Bulgaria

Asteromella silvarum Petr., Ann. Mycol. 23: 112, 1925.

Pycnidia epiphyllous, separated, arranged in rows between the veins of the leaves, subepidermal, then erumpent, globose, thick-walled, dark brown, 70–100 µm in diameter. Ostioles central, circular, 8–12 µm in diameter. Conidia rod-shaped, cylindrical, with rounded ends, straight, unicellular, hyaline, 2.5–4 × 1–1.5 µm.

Specimen examined — On leaves of *Carex atrata:* Rila Mts, 2550 m, 24 Sep 1955, leg. I. Bondev (ex SOM), fungus comm. & det. E. Sameva (SOMF 29 164).

Hendersonia culmicola Sacc., Michelia 1(2): 201, 1878.

Pycnidia gregarious between the veins of the leaves, immersed, then erumpent, globose or subglobose, pale brown or cinnamon-brown, 75–115 µm in diameter. Ostioles rounded, 10–15 µm in diameter, surrounded by dark brown cells. Conidia cylindrical, narrow clavate, straight or slightly curved, the base blunt or subtruncate, the apex rounded or pointed, with 3–6 distinct septa, some constricted at the septa, pale olivaceous, in mass brownish, 25–35 × (2.5–) 3–4 µm.

Specimen examined — On dried parts of leaves of *Festuca pirinica:* Pirin Mts, below Vihren peak (2900 m), 9 Aug 1938, leg. B. Achtarov (ex SOM), fungus comm. & det. E. Sameva (SOMF 29 165).

Melasmia mougeotii (Desm.) Arx, Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., 2de Reeks 51(3): 106, 1957.

Leaf spots black and spread out on both surfaces of leaves. Stromata amphigenous, mostly hypophyllous, crustaceous, flat, subepidermal, black, gregarious, discrete, sometimes confluent in clusters, multilocular; circular, 1–2 mm in diameter, or oblong and irregular, 3–5 × 1–2 mm. Loculi rounded, 125–250 µm in diameter. Conidiophores filiform, straight, hyaline, 20–30 × 1 µm. Conidia numerous, cylindrical with rounded ends, straight or slightly curved, unicellular, hyaline, (3.5–)4–6(–7) × 1.5–2(–2.5) µm.

Specimens examined — On leaves of *Bartsia alpina:* Pirin Mts, below Vihren peak, 2009, leg. V. Vladimirov, det. E. Sameva (SOMF 29 168); ditto, 11 Aug 2010, leg. H. Pedashenko & I. Apostolova, det. E. Sameva (SOMF 29 169).

Septoria macropoda Pass., Fungi Parm. Septor.: no. 141, 1879.

Pycnidia mainly epiphyllous, scattered or arranged in irregular lines, globose or slightly flattened, semi-imersed, thin-walled, brown, 55–125 µm in

diameter. Ostioles rounded or somewhat ellipsoidal, 15–25 µm in diameter. Conidia filiform, straight or curved, mostly with obtuse ends, some slightly tapered at the apex, unicellular or with 2–3 indistinct septa, hyaline, 20–40 × 1–1.5 µm.

Specimen examined — On leaves of *Poa alpina:* Rila Mts, Skakavitsa, 20 Aug 1956, leg. S. Petrov (ex SOM), fungus comm. & det. E. Sameva (SOMF 29 166).

Septoria tenella Cooke & Ellis, Grevillea 8(45): 11, 1879.

Pycnidia spread over both leaf surfaces, gregarious or forming indistinct rows, globose or subglobose, immersed, thick-walled, dark brown, 120–220 µm in diameter. Ostioles indistinct. Conidia filiform, straight, curved to arch-shaped or slightly flexuous, gradually tapered towards the both ends, subacute at the apex, unicellular, hyaline, 22–100 (–109) × 1–1.5 (–2) µm.

Specimen examined — On leaves of *Festuca pirinica:* Pirin Mts, below Vihren peak, 2900 m, 9 Aug 1938, leg. B. Achtarov (ex SOM), fungus comm. & det. E. Sameva (SOMF 29 165).

A smut fungus new for Bulgaria

Microbotryum silenes-acaulis M. Lutz et al., Mycol. Res. 112: 1289, 2008.

An anthericolous smut fungus on *Silene acaulis* has been reported from Bulgaria by Klika (1926) and Denchev (2001), as a species of *Ustilago* or *Microbotryum,* respectively. In 2008, a new cryptic species of *Microbotryum* on *Silene acaulis* was described as *M. silenes-acaulis.* Most probably, the following records refer to this cryptic species and therefore, it is considered here as a new species for Bulgaria.

Literature records — On *Silene acaulis:* Rila Mts, below Mussala peak, ca. 2900 m, J. Klika (Klika 1926, as *Ustilago violacea;* Denchev 2001, as *Microbotryum violaceum* s. lat.).

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Two new *Ceratocystis* species associated with mango disease in Brazil

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Abstract —*Mangifera indica,* a disease known as mango blight, murcha or seca da mangueira in Brazil, is caused by the canker wilt pathogen *Ceratocystis fimbriata* sensu lato. It is also closely associated with infestation by the non-native wood-boring beetle *Hypocryphalus mangiferae* (*Coleoptera: Scolytinae*). The aim of this study was to characterize *Ceratocystis* isolates obtained from diseased mango trees in Brazil. Identification was based on sequence data from ITS1+5.8S+ITS2 rDNA, part of the Beta-tubulin 1 gene, and part of the Transcription Elongation Factor 1-alpha gene. The Brazilian isolates grouped in two well defined and unique clades within *C. fimbriata* s.l. These were also distinct from *C. manginecans*, which causes a similar disease associated with *H. mangiferae* in Oman and Pakistan. Based on sequence comparisons and morphological characteristics, isolates representing the two phylogenetic clades are described as *C. mangicola* sp. nov. and *C. mangivora* sp. nov.

Key words — agricultural crop, bark beetles

Introduction

A disease typified by wilting of the leaves, flowers, and stems of mango trees (*Mangifera indica* L. (*Anacardiaceae*) mango) was first reported from Brazil in the 1930's (Viégas 1960, Ploetz 2003). The disease, commonly referred to as "mango blight", "seca" or "murcha da mangueira", represents one of the most important constraints to mango production in Brazil (Ploetz 2003). The causal agent of this disease was identified as *Ceratocystis fimbriata* Ellis & Halst. sensu lato (s.l.) (Viégas 1960, Piza 1966, Ribeiro 1980).

Ceratocystis fimbriata s.l. was first recognized as possibly encompassing more than one taxon by Webster & Butler (1967a, b), who showed host specificity

amongst isolates of the fungus. Isolates of *C. fimbriata* s.l. are morphologically similar, but many can be differentiated through DNA sequence analyses. During the past decade, numerous new and cryptic species in the *C. fimbriata* complex have been described. Examples include the African fungus *C. albifundus* M.J. Wingf. et al. (Wingfield et al. 1996, Barnes et al. 2005), *C. larium* M. van Wyk & M.J. Wingf. (Van Wyk et al. 2009a), *C. cacaofunesta* Engelbr. & T.C. Harr. (Engelbrecht & Harrington 2005), *C. fimbriatomima* M. van Wyk & M.J. Wingf. (Van Wyk et al. 2009b), *C. curvata* M. van Wyk & M.J. Wingf., *C. ecuadoriana* M. van Wyk & M.J. Wingf., and *C. diversiconidia* M. van Wyk & M.J. Wingf. (Van Wyk et al. 2011). In the strict sense, *C. fimbriata* is restricted to those isolates related to the sweet potato black-rot pathogen, first described by Halsted (1890) from diseased *Ipomoea batatas* (L.) Lam. (*Convolvulaceae*) (sweet potato) tubers in the USA (Engelbrecht & Harrington 2005). An alternative view is that phylogenetically different *C. fimbriata* s.l. isolates from various Brazilian hosts might represent populations of *C. fimbriata* s.s. rather than discrete taxa (Ferreira et al. 2010).

Ceratocystis species require wounds to infect trees (De Vay et al. 1963, Kile 1993). In Brazil, mango blight is closely associated with the wood-boring beetle *Hypocryphalus mangiferae* Stebbing (*Coleoptera: Scolytinae*) that is native to southern Asia (Wood 1982, Butani 1993, Atkinson & Peck 1994). It has been hypothesized that this insect aids in the dissemination of the fungus in Brazil (Ribeiro 1980, Yamashiro & Myazaki 1985, Ploetz 2003). Interestingly, the same beetle is associated with *Ceratocystis manginecans* M. van Wyk et al. that causes a serious disease of Mango trees in Oman and Pakistan (Al Adawi et al. 2006, Van Wyk et al. 2005; 2007a) and that has the same symptoms as mango blight in Brazil.

When Van Wyk et al. (2007a) described *C. manginecans,* only two *C. fimbriata* s.l. isolates from diseased mango in Brazil were included. These isolates differed phylogenetically from *C. manginecans* but were not treated as novel due to the small number of isolates available. Recently, a larger collection of *C. fimbriata* s.l. isolates associated with mango blight in Brazil has become available for study. The aim of this investigation was to compare these isolates with *C. manginecans* and thus to determine their identity.

Materials and methods

Isolates

A total of 15 isolates (Table 1) from diseased Mango trees obtained in Sao Paulo State in Brazil were transferred to 2% Malt Extract Agar (MEA) (20 g/L) (Biolab, Midrand, South Africa) and maintained at room temperature (~25°C). All cultures used are maintained in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI, University of Pretoria, South Africa). Representative

isolates have also been deposited with the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands). Cultures of representative isolates bearing fruiting structures of the fungi were dried on 30% glycerol and have been deposited with the National Collection of Fungi (PREM), South Africa.

Table 1. *Ceratocystis* spp. for which isolates or sequences were used in this study.

Species	Isolate .	GenBank acc. #	Host	Origin
C. acaciivora	CMW22563	EU588656, EU588636, EU588646	*Acacia mangium*	Indonesia
	CMW22564	EU588657, EU588637, EU588647	*A. mangium*	Indonesia
C. albifundus	CMW4068	DQ520638 EF070429 EF070400	*A. mearnsii*	RSA
	CMW5329	AF388947 DQ371649 EF070401	*A. mearnsii*	Uganda
C. atrox	CMW19383 CBS120517	EF070414 EF070430 EF070402	*Eucalyptus grandis*	Australia
	CMW19385 CBS120518	EF070415 EF070431 EF070403	*E. grandis*	Australia
C. cacaofunesta	CMW15051 CBS152.62	DQ520636 EF070427 EF070398	*Theobroma cacao*	Costa Rica
	CMW14809 CBS115169	DQ520637 EF070428 EF070399	*T. cacao*	Ecuador
C. colombiana	CMW9565 CBS121790	AY233864 AY233870 EU241487	soil	Colombia
	CMW5751 CBS121792	AY177233 AY177225 EU241493	*Coffea arabica*	Colombia
	CMW9572	AY233863 AY233871 EU241488	*Citrus reticulata*	Colombia
C. caryae	CMW14793 CBS114716	EF070424 EF070439 EF070412	*Carya cordiformis*	USA
	CMW14808 CBS115168	EF070423 EF070440 EF070411	*C. ovata*	USA
C. curvata	CMW22442 CBS122603	FJ151436 FJ151448 FJ151470	*Eucalyptus deglupta*	Colombia
	CMW22435 CBS122604	FJ151437 FJ151449 FJ151471	*E. deglupta*	Colombia
C. diversiconidia	CMW22445 CBS123013	FJ151440 FJ151452 FJ151474	*Terminalia ivorensis*	Colombia
	CMW22446	FJ151443 FJ151455 FJ151477	*T. ivorensis*	Colombia

Table 1, continued.

Species	Isolate No.	GenBank Acc. #	Host	Origin
C. ecuadoriana	CMW22092	FJ151432	*E. deglupta*	Colombia
	CBS124020	FJ151444		
		FJ151466		
	CMW22093	FJ151433	*E. deglupta*	Colombia
	CBS124021	FJ151445		
		FJ151467		
C. fimbriata s.s.	CMW15049	DQ520629	*Ipomaea batatas*	USA
	CBS141.37	EF070442		
		EF070394		
	CMW1547	AF264904	*I. batatas*	Papua New
		EF070443		Guinea
		EF070395		
C. fimbriata s.l.	C1345	AY157966	*Eucalyptus* sp.	Brazil
	C1987	AY585344	*Eucalyptus* sp.	Brazil
	C2041	AY585345	*Acacia mearnsii*	Brazil
	CMW14811	AY526288	*Colocasia esculenta*	Brazil
	CBS115171			
	C1905			
	CMW14791	AY526286	*C. esculenta*	Brazil
	CBS114713			
	C1865			
	C1900	AY526287	*C. esculenta*	Brazil
	C2032	AY526289	*C. esculenta*	Brazil
	C925	AY157967	*Gmelina arborea*	Brazil
	CMW14806	AY526292	*Ficus carica*	Brazil
	CBS115166			
	C1782			
	CMW14796	AY526307	*Colocasia esculenta*	USA, Hawaii
	CBS114720			
	C1715			
	CMW14804	AY526306	*C. esculenta*	USA, Hawaii
	CBS115164			
	C1714			
	BPI596162	AY526305	*C. esculenta*	China
	C1558	AY157965	*Mangifera indica*	Brazil
C. fimbriatomima	CMW24174	EF190963	*Eucalyptus* sp.	Venezuela
	CBS121786	EF190951		
		EF190957		
	CMW24176	EF190964	*Eucalyptus* sp.	Venezuela
	CBS121787	EF190952		
		EF190958		
C. larium	CMW25434	EU881906	*Styrax benzoin*	Indonesia
	CBS122512	EU881894		
		EU881900		
	CMW25435	EU881907	*S. benzoin*	Indonesia
	CBS122606	EU881895		
		EU881901		
C. mangicola	**CMW14797**	**AY953382**	***Mangifera indica***	**Brazil**
	CBS114721	**EF433307**		
	C1688	**EF433316**		
	CMW27306	**FJ200256**	***M. indica***	**Brazil**
		FJ200269		
		FJ200282		
	CMW28907	**FJ200257**	***M. indica***	**Brazil**
		FJ200270		
		FJ200283		
	CMW28908	**FJ200258**	***M. indica***	**Brazil**
		FJ200271		
		FJ200284		

Table 1, continued.

Species	Isolate No.	GenBank Acc. #	Host	Origin
(*C. mangicola*)	**CMW28913**	**FJ200259**	***M. indica***	**Brazil**
		FJ200272		
		FJ200285		
	CMW28914	**FJ200260**	***M. indica***	**Brazil**
		FJ200273		
		FJ200286		
C. manginecans	CMW13851	AY953383	*M. indica*	Oman
	CBS121659	EF433308		
		EF433317		
	CMW13852	AY953384	*Hypocryphalus*	Oman
	CBS121660	EF433309	*mangiferae*	
		EF433318		
	CMW23634	EF433302	*M. indica*	Pakistan
	CMW23628	EF433303	*M. indica*	Pakistan
C. mangivora	**CMW15052**	**EF433298**	***M. indica***	**Brazil**
	CBS600.70	**EF433306**		
	C74	**EF433315**		
	CMW27304	**FJ200261**	***M. indica***	**Brazil**
	CBS127204	**FJ200274**		
		FJ200287		
	CMW27305	**FJ200262**	***M. indica***	**Brazil**
	CBS128340	**FJ200275**		
		FJ200288		
	CMW27307	**FJ200263**	***M. indica***	**Brazil**
		FJ200276		
		FJ200289		
	CMW28909	**FJ200264**	***M. indica***	**Brazil**
		FJ200277		
		FJ200290		
	CMW28910	**FJ200265**	***M. indica***	**Brazil**
		FJ200278		
		FJ200291		
	CMW28911	**FJ200266**	***M. indica***	**Brazil**
		FJ200279		
		FJ200292		
	CMW28912	**FJ200267**	***M. indica***	**Brazil**
		FJ200280		
		FJ200293		
	CMW28916	**FJ200260**	***M. indica***	**Brazil**
		FJ200281		
		FJ200294		
C. neglecta	CMW17808	EF127990	*Eucalyptus* sp.	Colombia
	CBS121789	EU881898		
		EU881904		
	CMW18194	EF127991	*Eucalyptus* sp.	Colombia
	CBS121017	EU881899		
		EU881905		
C. obpyriformis	CMW23807	EU245004	*Acacia mearnsii*	South Africa
	CBS122608	EU244976		
		EU244936		
	CMW23808	EU245003	*A. mearnsii*	South Africa
	CBS122511	EU244975		
		EU244935		
C. papillata	CMW8857	AY233868	*Annona muricata*	Colombia
		AY233878		
		EU241483		
	CMW8856	AY233867	*Citrus limon*	Colombia
	CBS121793	AY233874		
		EU241484		

Table 1, continued.

Species	Isolate No.	GenBank Acc. #	Host	Origin
(*C. papillata*)	CMW10844	AY177238 AY177229 EU241481	*Coffea arabica*	Colombia
C. pirilliformis	CMW6569	AF427104 DQ371652 AY528982	*Eucalyptus nitens*	Australia
	CMW6579 CBS118128	AF427105 DQ371653 AY528983	*E. nitens*	Australia
C. platani	CMW14802 CBS115162	DQ520630 EF070425 EF070396	*Platanus occidentalis*	USA
	CMW23918	EF070426 EF070397 EU426554	*Platanus* sp.	Greece
C. polychroma	CMW11424 CBS115778	AY528970 AY528966 AY528978	*Syzygium aromaticum*	Indonesia
	CMW11436 CBS115777	AY528971 AY528967 AY528979	*S. aromaticum*	Indonesia
C. polyconidia	CMW23809 CBS122289	EU245006 EU244978 EU244938	*Acacia mearnsii*	South Africa
	CMW23818 CBS122290	EU245007 EU244979 EU244939	*A. mearnsii*	South Africa
C. populicola	CMW14789 CBS119.78	EF070418 EF070434 EF070406	*Populus* sp.	Poland
	CMW14819 CBS114725	EF070419 EF070435 EF070407	*Populus* sp.	USA
C. smalleyi	CMW14800 CBS114724	EF070420 EF070436 EF070408	*Carya cordiformis*	USA
	CMW26383 CBS114724	EU426553 EU426555 EU426556	*C. cordiformis*	USA
C. tanganyicensis	CMW15991 CBS122295	EU244997 EU244969 EU244929	*A. mearnsii*	Tanzania
	CMW15999 CBS122294	EU244998 EU244970 EU244939	*A. mearnsii*	Tanzania
C. tsitsikammensis	CMW14276 CBS121018	EF408555 EF408569 EF408576	*Rapanea melanophloeos*	South Africa
	CMW14278 CBS121019	EF408556 EF408570 EF408577	*R.. melanophloeos*	South Africa
C. variospora	CMW20935 CBS114715	EF070421 EF070437 EF070409	*Quercus alba*	USA
	CMW20936 CBS114714	EF070422 EF070438 EF070410	*Q. robur*	USA

TABLE 1, concluded.

SPECIES	ISOLATE NO.	GENBANK ACC. #	HOST	ORIGIN
C. virescens	CMW11164	DQ520639 EF070441 EF070413	*Fagus americana*	USA
	CMW3276	AY528984 AY528990 AY529011	*Q. robur*	USA
C. zombamontana	CMW15235	EU245002 EU244974 EU244934	*Eucalyptus* sp.	Malawi
	CMW15236	EU245000 EU244972 EU244932	*Eucalyptus* sp.	Malawi

*CMW numbers are in the Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa; CBS numbers are in the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands; C numbers are in the T. Harrington collection Iowa State University, USA; BPI numbers are in the US National Fungus collection.

Phylogenetic comparisons

DNA was extracted from the isolates obtained from mango in Brazil according to Van Wyk et al. (2006). Three sets of analyses were run on DNA sequence data obtained from these isolates. The first dataset comprising the Internal Transcribed Spacer region 1 and 2 including the 5.8S rRNA operon (ITS) included sequences for all species in the *C. fimbriata* s.l. complex as well as most *C. fimbriata* sequences available in GenBank (http://ncbi.nlm.nih.gov) and from a variety of plants in Brazil. For the second dataset, three gene regions were targeted for PCR including the ITS, part of the Beta-tubulin 1 (βt) gene, and part of the Transcription Elongation Factor 1-alpha gene (EF1-α). Data for these three gene regions were combined. The third dataset consisted of only the isolates from Brazil and from mango with each gene region (ITS, βt, EF1-α) treated separately.

DNA amplification was achieved with the primer sets ITS1 and ITS4 (White et al. 1990), βt1a and βt1b (Glass & Donaldson 1995), and EF1F and EF1R (Jacobs et al. 2004), following the protocols described by Van Wyk et al. (2006). Amplification was assessed with the aid of gel electrophoresis in the presence of ethidium bromide. PCR amplicons were purified using 6% Sephadex G-50 columns (Steinheim, Germany) and sequenced in both directions using the ABI PRISM™ Big DYE Terminator Cycle Sequencing Ready Reaction Kit (Applied BioSystems, Foster City, California), with the same primers as those used for DNA amplification. Sequencing reactions were run on an ABI PRISM™ 3100 Autosequencer (Applied BioSystems, Foster City, California, USA).

Sequences were analysed using the software programme Chromas Lite 2.01 (http://www.technelysium.com.au). Sequence data obtained in this study for Brazilian isolates from mango were compared with those residing in the *C. fimbriata* s.l. clade for *Ceratocystis* obtained from GenBank or those previously published (Van Wyk et al. 2005, 2007a,b, 2009a,b, 2011). These sequences were aligned using MAFFT (http://timpani.genome.ad.jp/%7emafft/server/) (Katoh et al. 2002) and confirmed manually. Thereafter, the *C. fimbriata* s.l. dataset was analyzed using PAUP version 4.0b10* (Swofford 2002). Sequences for the three gene regions were analyzed separately and a partition homogeneity test (Swofford 2002) was used to determine whether the

three datasets (ITS, βt and EF1-α) could be combined. The combined analyses were run as described in Van Wyk et al. (2009b). Sequences derived from this study were deposited in GenBank (Table 1) and the accompanying datasets and trees are deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S11630).

A modeltest (MrModeltest2) was run on each gene region to determine nucleotide substitution rates (Nylander 2004) for incorporation into Bayesian analyses (MrBayes version 3.1.1) to determine whether nodes obtained with PAUP had statistical support (Ronquist & Huelsenbeck 2003). One million trees were generated using the Markov Chain Monte Carlo (MCMC) procedure. Four chains, two hot and two cold, were utilized to obtain the results. Trees were sampled every 100th generation and printed. Tree likelihood scores were assessed to determine the number of trees that had formed before stabilization to avoid including trees that had formed before convergence. Trees outside the point of convergence were discarded by means of the burn-in procedure (Ronquist & Huelsenbeck 2003).

Molecular Evolutionary Genetics Analysis (MEGA) 4 (Tamura et al. 2007) was used to determine the level of variation between the isolates from a wide range of hosts in Brazil for the ITS region only. In addition, this approach was applied for the ITS, βt, and EF1-α for the isolates obtained from mango trees in Brazil and including *C. manginecans* previously described from Oman and Pakistan. Sequences for each of the three gene regions were inspected to determine the number of fixed alleles between them.

An allele network was drawn using the software TCS (Clement et al. 2000). The dataset consisted of the combined gene regions (ITS, βt and EF1-α) of all the isolates obtained from mango in Brazil and including *C. manginecans* and *C. fimbriatomima*.

Culture characteristics and morphology

Based on the phylogenetic comparisons, two groups (B1 and B2) of isolates emerged. Three representatives from each of the two groups (CMW14797, CMW27306, CMW28907 and CMW15052, CMW27304, CMW27305) were randomly selected for growth studies in culture at different temperatures. The isolates were grown for 14 days on 2% MEA, after which 5mm diam. plugs were transferred to the centers of 90mm Petri plates containing 2% MEA. These plates were incubated at temperatures between 5 and 35°C at five degree intervals. Five plates were used for each isolate at each of the test temperatures and the entire experiment was repeated once. The colony colours for isolates were assigned using the colour charts of Rayner (1970).

For microscope studies, the same six isolates, representing the two groups (B1 and B2) that were used to compare culture characteristics were selected. These cultures were grown for 10 days on 2% MEA plates. Fungal structures were selected and mounted in lactic acid on glass slides. Photographic images were captured with a Carl Zeiss compound microscope and using a Zeiss Axio Vision camera system. For isolates CMW14797 and CMW28305, 50 measurements were made for taxonomically relevant morphological characteristics, while 10 measurements were taken for isolates CMW27306, CMW28907, CMW15052 and CMW27304. The averages and standard deviations (stdv) were computed for all the measurements that are presented in the descriptions as (minimum–) mean minus stdv – mean plus stdv (–maximum). Where the minimum value was the same as the mean minus the stdv, a parenthetical minimum was not included.

Results

Phylogenetic comparisons

ITS sequences for species in *C. fimbriata* s.l. —including unidentified isolates from *Colocasia* (*Araceae*) (taro), *Mangifera* (*Anacardiaceae*) (mango), *Gmelina* (*Lamiaceae*) (yemane) and *Ficus* (*Moraceae*) (fig) in Brazil— gave a 614 bp dataset for 83 isolates. This dataset consisted of 234 constant, 11 parsimony uninformative, and 369 parsimony informative characters. Of the five trees obtained in these analyses, one was selected for presentation (Fig. 1). The tree had the following characteristics; tree length = 1279, Consistency Index = 0.6, Rescaled Index = 0.5, Retention Index = 0.9.

MrModeltest2 selected the GTR+I+G model for the ITS gene region. These settings were included in the Bayesian analyses and four thousand trees were discarded because they were obtained outside the point of convergence. The Bayesian probabilities obtained in MrBayes were included in the phylogram (Fig. 1) obtained in PAUP. The probabilities obtained in the Bayesian analyses were similar to the support values obtained in PAUP.

The isolates from Brazil grouped into several polyphyletic clades (Fig. 1). These included a well-supported clade (Bootstrap 86%) represented by two isolates, one from *Acacia* (*Mimosaceae*) the other from *Eucalyptus* (*Myrtaceae*) A second clade included only isolates from taro (Bootstrap 85%). Isolates from mango and a *Eucalyptus* and *Gmelina* isolate resided in a discrete clade (Bayesian 91%), and *C. manginecans* isolates grouped in a clade sister to that clade (Bootstrap 87%). A group of isolates from mango resided in a clade with strong support (Bootstrap 99%, Bayesian 86%), and an isolate from fig was sister to that clade.

Amplicons of ~500 bp (ITS and β-tubulin) and ~800 bp (EF1-α) were obtained for the Brazilian isolates of *C. fimbriata* s.l. from mango (Table 1). The PHT resulted in a low P-value (P=0.01), possibly due to the small amount of variation in the βt gene region. Although the P-value was low, this value remained acceptable (Sullivan 1996, Cunningham 1997) to support combination of the data for the three gene regions. The combined dataset for the three gene regions consisted of a total of 1971 characters, 1066 of which were constant, 57 were parsimony-uninformative, and 848 were parsimony informative. Twenty-two most parsimonious trees were obtained, one of which (Fig. 2) was selected for presentation (Tree length = 2361, Consistency Index = 0.6, Rescaled Index = 0.5, Retention Index = 0.9).

MrModeltest2 selected the GTR+I+G model for the ITS gene region, the GTR+G model for the βt gene region and the HKY+I+G for the EF1-α gene region. These settings were included in the Bayesian analyses. Two thousand trees were discarded as they were outside of the point of convergence. The posterior probabilities for the branch nodes were included in the tree obtained

with PAUP (Fig. 2). The posterior probabilities supported the bootstrap values obtained using PAUP.

The isolates from mango in Brazil grouped in two distinct clades (B1 and B2), with high bootstrap support (100% and 100%, respectively). These two phylogenetic groups were sister to *C. manginecans*, the species most closely related to them. All other species considered in this study, formed well-supported and distinct clades, confirming their unique nature.

The single ITS gene tree (Fig. 3) had a structure similar to the tree based on combined sequences for the three gene regions. The two groups of isolates from mango (B1 and B2) in Brazil grouped apart from *C. fimbriatomima* and *C. manginecans* with high bootstrap support. The single gene trees for the βt and EF1-α gene region did not distinguish between the two groups of isolates from mango in Brazil but they did distinguish *C. fimbriatomima* and *C. manginecans* from these two groups.

The number of fixed alleles between the four groups (three from mango and one from *Eucalyptus*), *C. manginecans,* the two groups of isolates obtained from Mango in Brazil (Group B1 and Group B2) and *C. fimbriatomima* varied within and between groups (Table 2). Analysis of the combined dataset for the three gene regions using TCS resulted in two allele trees (Fig. 4). *Ceratocystis fimbriatomima* was represented on its own while all three taxa from diseased mango including *C. manginecans* and the two groups identified in this study, resided in a single allele tree.

Table 2. Comparison of differing sequences and number of fixed alleles in *Ceratocystis* spp. from mango and the closely related species *C. fimbriatomima*. Shaded cells indicate variations within each species.

ITS	*C. mangicola*	*C. mangivora*	*C. manginecans*	*C. fimbriatomima*
C. mangicola	**4**	16	6	10
C. mangivora	16	**2**	20	14
C. manginecans	6	20	**0**	14
C. fimbriatomima	10	14	14	**1**

βt	*C. mangicola*	*C. mangivora*	*C. manginecans*	*C. fimbriatomima*
C. mangicola	**1**	0	5	8
C. mangivora	0	**3**	4	7
C. manginecans	5	4	**0**	3
C. fimbriatomima	8	7	3	**1**

EF-1α	*C. mangicola*	*C. mangivora*	*C. manginecans*	*C. fimbriatomima*
C. mangicola	**1**	0	1	0
C. mangivora	0	**9**	1	0
C. manginecans	1	1	**0**	1
C. fimbriatomima	0	0	1	**0**

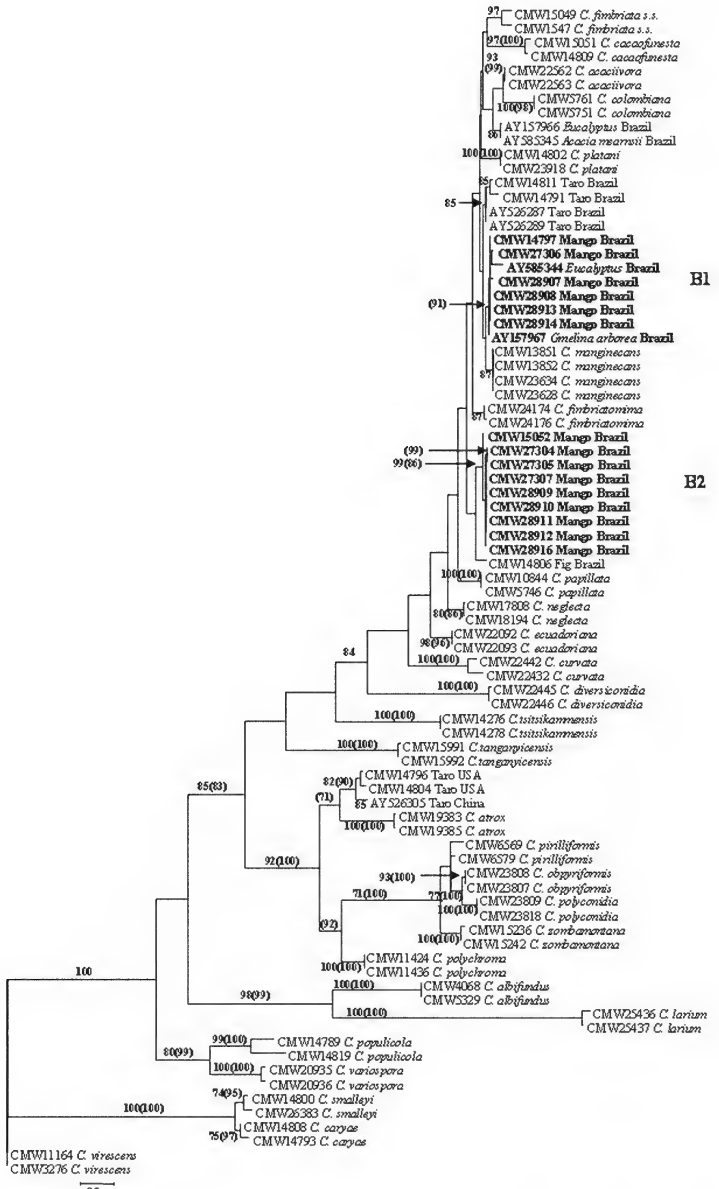

FIGURE 1. Phylogenetic tree based of the ITS gene region for *Ceratocystis mangicola* (B1), *C. mangivora* (B2), and other species in the *C. fimbriata* s.l. complex including isolates from Brazil obtained from various hosts. Bootstrap values are indicated at the branches, with Bayesian support in brackets.

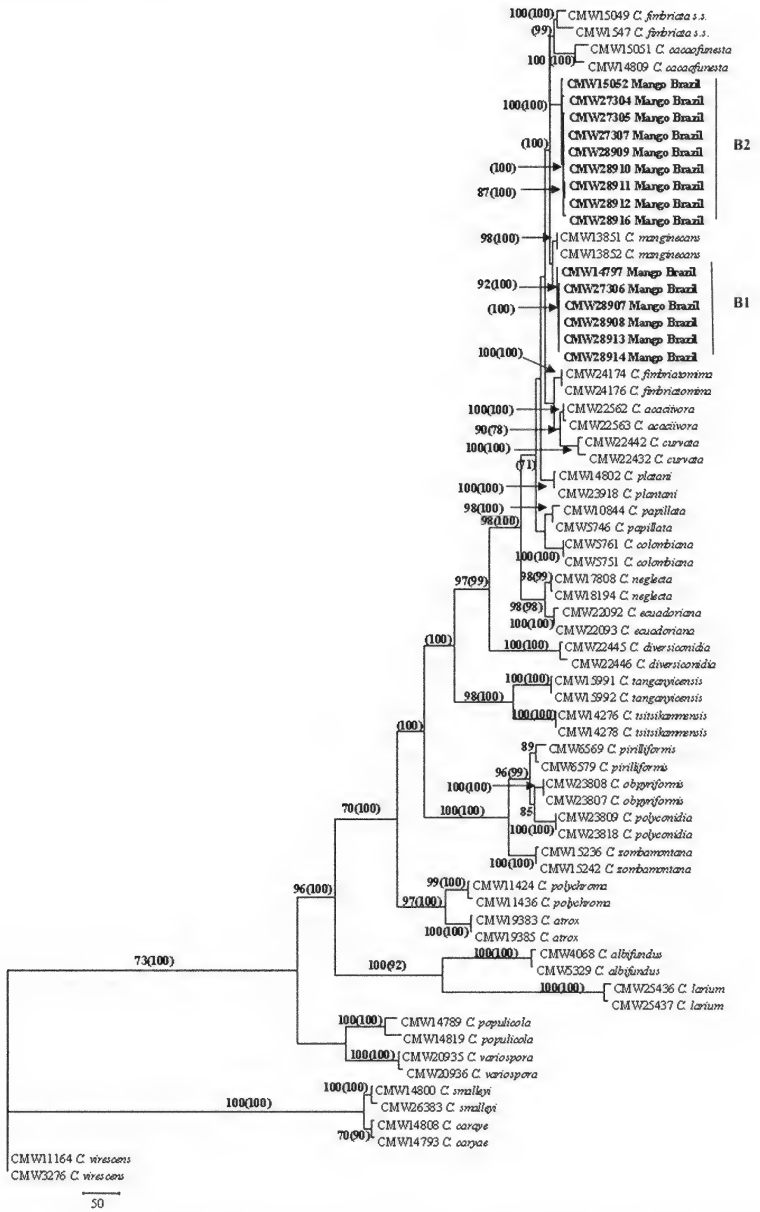

FIGURE 2. Phylogenetic tree based on the combined regions of the ITS, β-tubulin and EF1-α for *Ceratocystis mangicola* (B1), *C. mangivora* (B2), and other species in the *C. fimbriata* s.l. species complex. Bootstrap values are indicated at the branches, with Bayesian support in brackets.

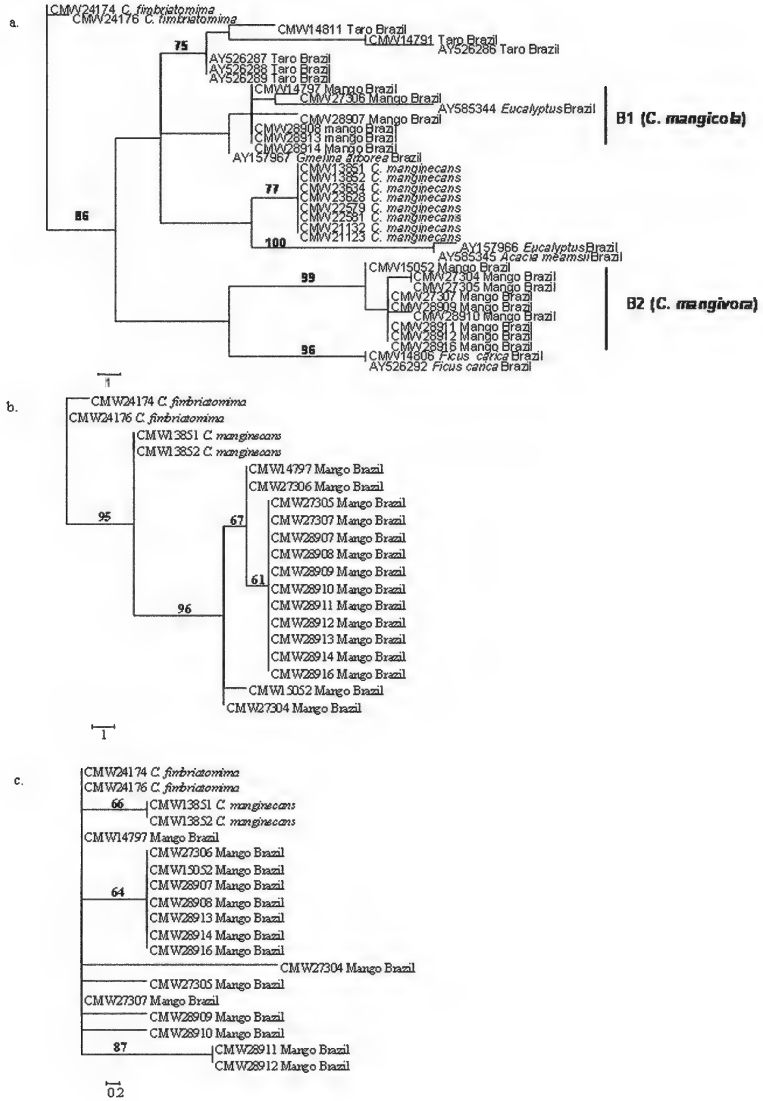

FIGURE 3. Three separate unrooted phylogenetic trees representing three gene regions. Isolates representing the two groups from Brazil mango as well as *Ceratocystis manginecans* and *C. fimbriatomima* were included. a. ITS. b. βt. c. EF1-α.

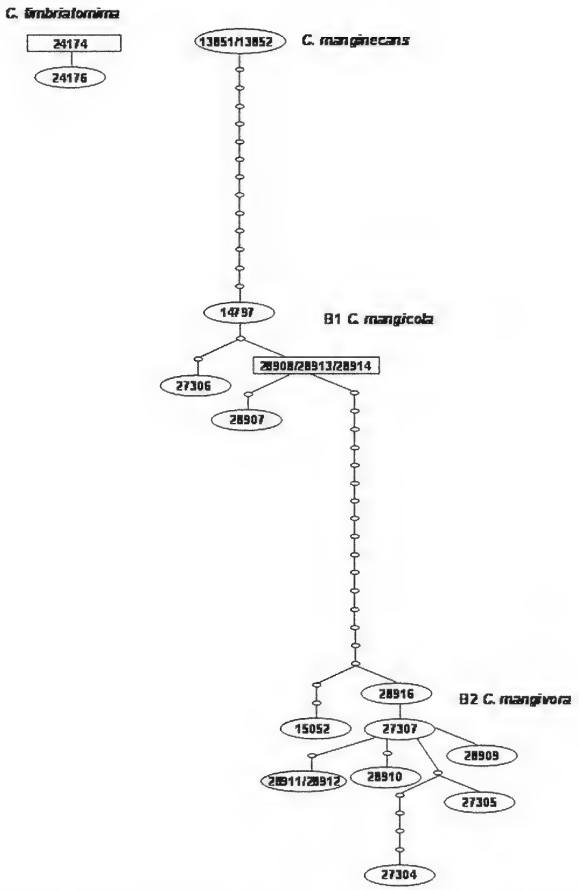

FIGURE 4. An allele network of the two groups of isolates from Brazil as well as a closely related species *C. manginecans* also isolated from mango trees and *Ceratocystis fimbriatomima*. The numbers represent CMW numbers (TABLE 1).

Culture characteristics and morphology

Isolates representing Group B1 were morphologically similar to other species in *C. fimbriata* s.l. They produced a banana odour, typical of fungi in this group. After 2 weeks on 2% MEA, the colonies had a dark brown (snuff brown, 15"k) colour (Rayner 1970) with large numbers of perithecia visible on the surface of the cultures. At 5°C and 35°C, no growth was observed after 7 days. At 10°C (8 mm), 15°C (22 mm), 20°C (36 mm) and 30°C (20 mm) diminished growth was observed after 7 days while the optimum temperature for growth of these isolates was 25°C (44 mm).

Isolates residing in Group B2 were similar to those in Group B1 producing a banana odour and they had a similar morphology. After 2 weeks on 2% MEA, the colonies also had a dark brown (snuff brown, 15"k) colour (Rayner 1970) with many perithecia produced on the culture surface. As with the isolates in Group B1, after seven days, there was no growth at either 5 or 35°C. Some growth was observed at the other temperatures tested 10°C (7 mm) and 15°C (22 mm), 20°C (41 mm), 30°C (36 mm) and 25°C (45 mm) represented the optimum temperature for growth.

Taxonomy

Based on DNA sequence comparisons and (to a lesser extent) morphology, isolates from mango in Brazil could be separated into two distinct groups. These groups represent previously unknown species that are described as follows:

Ceratocystis mangicola M. van Wyk & M.J. Wingf., **sp. nov.** FIGURE 5

MYCOBANK MB511886

Hyphae ostiolares hyalinae divergentes convergentesque, (47–)57–73(–79) µm longa. Conidiophorae biformes; primariae phialidicae, lageniformes, hyalinae; secondariae copiosae, tubiformes, apicibus expansis, hyalinae.

TYPE: Brazil, São Paulo State, from diseased *Mangifera indica* trees, isolated C.J. Baker C1688, 2000. (**holotype** PREM60182 (culture dried on 30% glycerol); culture ex-type CMW14797 = CBS114721).

ETYMOLOGY: The epithet refers to the fact that the fungus occurs on mango.

Colonies brown (15"k) on 2% MEA. Odour banana. Hyphae smooth and segmented. Ascomatal bases globose to sub-globose, dark-brown to black, (125–)139–199(–230) µm wide, (115–)136–192(–236) µm long. Ascomatal necks brown becoming lighter towards apices (541–)766–980(–1103) µm long, (21–)26–36(–46) µm wide at base, (15–)19–27(–33) µm wide at tip. Ostiolar hyphae of two types; hyaline, divergent and convergent, (47–)57–73(–79) µm long. Asci evanescent, not seen. Ascospores hyaline, hat-shaped, 3–4 µm long, 3–4 µm wide excluding sheath, 5–6 µm wide including sheath.

Thielaviopsis ANAMORPH: Conidiophores of two morphological forms. Primary conidiophores phialidic, lageniform, hyaline, (59–)71–119(–140) µm long, (3–)4–6(–7) µm wide at base, 5–7(–8) µm wide at broadest point, 3–5(–8) µm wide at tips. Secondary conidiophores, abundant, tube-like, flaring at apices, hyaline, (53–)72–114(–148) µm long, 4–6(–7) µm wide at bases and 6–8(–9) µm wide at tips. Conidia of two types. Primary conidia, hyaline, cylindrical, (15–)18–24(–29) µm long, (3–)4–6 µm wide. Secondary conidia, abundant, hyaline, barrel to sub-globose shaped, (6–)7–9(–11) µm long, 6–8 µm wide. Chlamydospores rare, brown, thick-walled, globose to sub-globose, (12–)14–16(–17) µm long by (9–)11–13(–14) µm wide.

Figure 5. Morphological characteristics of *Ceratocystis mangicola* (from holotype): a. Globose ascomata. b. Divergent ostiolar hyphae. c. Convergent ostiolar hyphae. d. Primary phialidic conidiophore. e. Secondary conidiophore with emerging chain of barrel-shaped conidia. f. Dark colored chlamydospores. g. Hat-shaped ascospores. h. Cylindrical conidia. i. Chain of barrel-shaped conidia. Scale bars: a = 100 µm, b–c, e–f, h–i = 10 µm, d = 20 µm, g = 5 µm.

HABITAT & DISTRIBUTION: Isolated from *Mangifera indica* trees and associated with the wood-boring scolytine *Hypocryphalus mangiferae*. Known from São Paulo State, North West Brazil.

ADDITIONAL SPECIMENS EXAMINED: **BRAZIL. SÃO PAULO STATE, VOTUPORANGA**, from diseased *Mangifera indica* trees, isolated C.J. Rossetto 13750-1, 2007, PREM60183, living culture CMW27306; isolated C.J. Rossetto 13959, 2008, PREM60184, living culture CMW28907; isolated C.J. Rossetto 13911, 2008, PREM60186, living culture CMW28913; **SÃO PAULO STATE, SANTA BÁRBARA D'OESTE**, from diseased *Mangifera indica* trees isolated C.J. Rossetto 13977, 2008, PREM60185, living culture CMW28908; **SÃO PAULO STATE, PINDORAMA**, from diseased *Mangifera indica* trees isolated C.J. Rossetto 13966, 2008, PREM60187, living culture CMW28914.

NOTES: *Ceratocystis mangicola* (B1; CMW14797) is distinguished from all other species in the *C. fimbriata* s.l. complex based primarily on phylogenetic inference. However, it also has ostiolar hyphae that are both divergent and convergent as opposed to being only divergent in most species of this genus.

Ceratocystis mangivora M. van Wyk & M.J. Wingf., **sp. nov.** FIGURE 6

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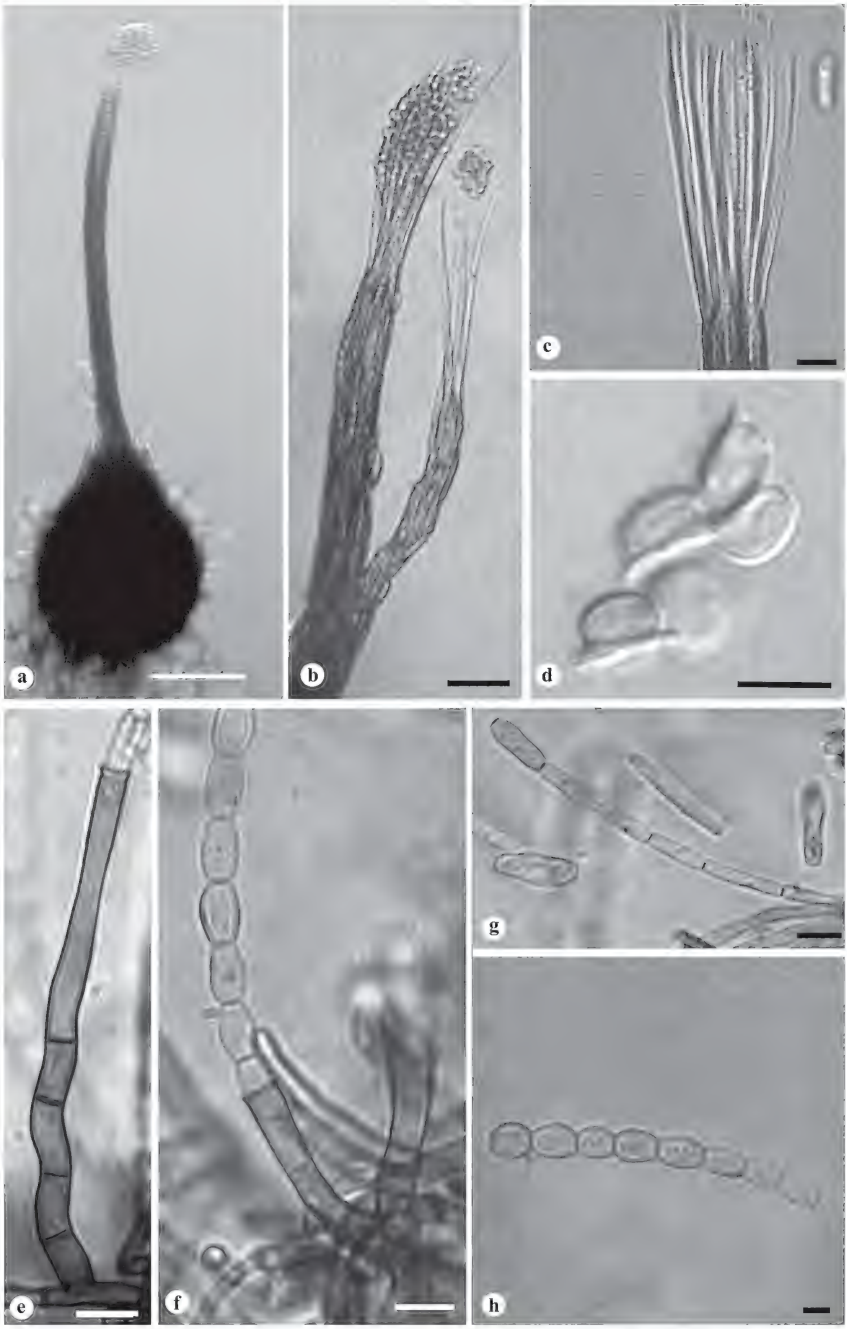
Bases ascomatum globosae vel obpyriformes. Colla ascomatum brunnea, apicem versus pallescentia, apice in duo vel plura ramose. Conidiophorae biformes; primariae phialidicae lageniformes hyalinae; secondariae abundantes tubiformes apice expansae hyalinae. Chlamydosporae absunt.

TYPE: Brazil. São Paulo State, Campinas, from diseased *Mangifera indica* trees, isolated C.J. Rossetto 12132, 2001. (**holotype** PREM60570 (culture dried on 30% glycerol); culture ex-type CMW27305 = CBS128340).

ETYMOLOGY: The epithet refers to the fact that the fungus causes a disease on mango.

Colonies brown (15"k) on 2% MEA. Odour banana. Hyphae smooth and segmented. Ascomatal bases globose to obpyriform, dark-brown to black, (171–)188–244(–295) µm wide, (174–)192–256(–310) µm long. Ascomatal necks brown becoming lighter towards apices, branching at apices into two or more necks, (394–)437–575(–654) µm long, (21–)26–34(–40) µm wide at base, (16–)19–29(–35) µm wide at tip. Ostiolar hyphae hyaline, divergent and convergent, (60–)75–91(–96) µm long. Asci evanescent, not seen. Ascospores hyaline, hat-shaped, 3–5 µm in length, 4–6 µm wide excluding sheath, 5–8 µm wide including sheath.

Thielaviopsis ANAMORPH: Conidiophores of two morphological forms. Primary conidiophores phialidic, lageniform, hyaline, (70–)78–106(–124) µm long, (3–)5–7 µm wide at base, 5–7(–8) µm wide at broadest point, 3–5 µm wide at tips. Secondary conidiophores, abundant, tube-like, flaring at apices, hyaline, (42–)62–100(–118) µm long, (3–)4–6 µm wide at bases and (4–)6–8(–9) µm wide at tips. Conidia of two types: Primary conidia, hyaline, cylindrical, (12–)16–24(–31) µm long, 2–5 µm wide. Secondary conidia, abundant, hyaline,


a
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barrel-shaped, (8–)9–13(–15) µm long, (5–)6–8(–9) µm wide. Chlamydospores absent.

Habitat & distribution: Isolated from *Mangifera indica* trees. Associated with the wood-boring scolytid *Hypocryphalus mangiferae*. Known from São Paulo State, Central East Brazil.

Additional specimens examined: **BRAZIL, São Paulo State,** from diseased *Mangifera indica* trees, isolated M. Barreto Figueiredo, 1970, PREM60188, living culture CMW15052 = CBS600.70; **BRAZIL, São Paulo State, Campinas,** from diseased *Mangifera indica* trees, isolated C.J. Rossetto 12093, 2001, PREM60189, living culture CMW27304 = CBS127204; isolated C.J. Rossetto 12036, 2001, PREM60190, living culture CMW27307; **BRAZIL, São Paulo State, Santa Bárbara d'Oeste,** from diseased *Mangifera indica* trees, isolated C.J. Rossetto 13988, 2008, PREM60191, living culture CMW28909; isolated C.J. Rossetto 13987, 2001, PREM60192, living culture CMW28910. **BRAZIL, São Paulo State, Tupi (near Piracicaba),** from diseased *Mangifera indica* trees, isolated C.J. Rossetto 13989, 2008, living culture CMW28911; isolated C.J. Rossetto 13989-1, 2008, living culture CMW28912; **BRAZIL, São Paulo State, Valinhos,** from diseased *Mangifera indica* trees, isolated C.J. Rossetto 13986, 2008, living culture CMW28916.

Notes: Isolates of *C. mangivora* (B2; CMW27305) can have ascomatal necks that branch dichotomously at the apices with ostiolar hyphae being either divergent or convergent. Isolates of this species also did not produce chlamydospores in culture.

Discussion

Results of this study showed that a relatively large collection of *Ceratocystis* isolates from mango trees suffering from Mango blight in Brazil reside in two distinct phylogenetic clades. These groups are, furthermore, distinct from *C. manginecans,* which causes a similar disease of mango in Oman and Pakistan (Al Adawi et al. 2006, Van Wyk et al. 2005, 2007a). The isolates residing in these two groups are consequently treated as distinct taxa and the names *C. mangicola* and *C. mangivora* have been provided for them.

The mango tree blight in Brazil, known for almost a century, was previously ascribed to *C. fimbriata* s.l., which we now recognize represents a relatively large number of cryptic taxa. These species are morphologically very similar, and although individual species can be distinguished from their closest relatives, recognition based solely on morphological characteristics would

Figure 6. Morphological characteristics of *Ceratocystis mangivora* (from holotype): a. Globose to obpyriform ascomata. b. Ascomatal neck branching into two apices with both convergent and divergent ostiolar hyphae. c. Convergent ostiolar hyphae. d. Hat-shaped ascospores. e. Primary conidiophore. f. Secondary conidiophore with emerging chain of barrel-shaped conidia. g. Chain of cylindrical conidia. h. Chain of barrel-shaped conidia. Scale bars: a = 100 µm, b–c, e–g = 10 µm, d, h = 5 µm.

be very difficult. This situation exists for many groups of fungi, for example *Fusarium* species in the *Gibberella fujikuroi* species complex (Leslie et al. 1992, O'Donnell et al. 2000). *Ceratocystis mangicola* is phylogenetically most closely related to *C. manginecans,* a known pathogen of mango and other crops in Oman and Pakistan. *Ceratocystis mangivora* (also described in this study) has no well-defined sister group but is also closely related to the other two mango pathogens. Isolates of these species were not only distinct from each other but also phylogenetically distinct from the mango pathogen, *C. manginecans*. The species phylogenetically most closely related to these mango pathogens from Brazil is *C. fimbriatomima*, which was first isolated from *Eucalyptus* trees in Venezuela (Van Wyk et al. 2009b).

Phylogenetic analyses of sequences for the ITS gene region strongly supported separation of *C. mangicola* and *C. mangivora*. In contrast, the βt and EF1-α gene regions for the single gene trees showed little or no variation between *C. mangicola* and *C. mangivora*. This is not uncommon for species in the *C. fimbriata* complex (Van Wyk et al. 2010). Similarly the allele trees for four species; *C. fimbriatomima* and the three mango pathogens, *C. manginecans*, *C. mangicola,* and *C. mangivora* showed that the three species from mango were most closely related to each other. This suggests a common ancestor for the three mango pathogens and the fact that they have probably undergone speciation relatively recently.

Ceratocystis mangicola and *C. mangivora* are morphologically very similar, both producing dark brown cultures with a banana odour that is characteristic of many species of *Ceratocystis.* However, isolates representing the two species could be distinguished from each other based on various micro-morphological characteristics. Thus, *C. mangicola* isolates have both divergent and convergent ostiolar hyphae, a characteristic not noted for other *C. fimbriata* s.l. species except *C. mangivora* described here. Isolates of *C. mangivora* consistently display branched ascomatal necks that give rise to either convergent or divergent ostiolar hyphae. Furthermore, similar to some species in *C. fimbriata* s.l., *C. mangivora* did not produce chlamydospores in culture while these structures are very obvious in cultures of *C. mangicola. Ceratocystis mangicola* isolates also have globose to sub-globose ascomatal bases compared to the globose to obpyriform bases in *C. mangivora.*

A previous study on *C. fimbriata* s.l. from different hosts (including mango in Brazil) treated the isolates as a genetically diverse population representing a single taxon (Ferreira et al. 2010). It would have been interesting to include data from that study's isolates in our own research, which might have provided a more robust species delimitation for *C. mangicola* and *C. mangivora.* The unavailability of the isolates for study and the absence of their sequence data from GenBank preclude comparisons at that level.

An interesting aspect of the mango blight disease in Brazil and in Oman is the fact that the pathogens are associated with the same wood-boring insect (*H. mangiferae*) in both areas of the world. *Hypocryphalus mangiferae* is a monophagous bark beetle found only on *Mangifera* species (Schedl 1961). Its source area is likely the same as mango trees in tropical Asia (Wood 1982, Butani 1993, Kostermans & Bompard 1993). Both tree and beetle have been introduced into Brazil (Wood 1982, Butani 1993, Kostermans & Bompard 1993).

Both *C. mangicola* and *C. mangivora* are suspected to be native to Brazil. As with most *Ceratocystis* species, a wound is required for *C. mangicola* and *C. mangivora* to infect mango trees (Silva et al. 1959). Intensive studies of diseased mango trees in Brazil have shown that *H. mangiferae* is the only insect present during the early disease stages. *Xyleborus* species (*Coleoptera: Curculionidae*) typically appear when the disease spreads down towards the larger branches and *Cerambycidae* only appear when the disease reaches the trunk regions (Silva et al. 1959, Castro 1960, Medeiros & Rossetto 1966, Rossetto et al. 1980). Studies have also shown that *H. mangiferae* is the primary vector of *C. mangicola* and *C. mangivora* in Brazil (Ribeiro & Rossetto 1971). A similar vector relationship has also been shown for *C. manginecans* in Oman (Al Adawi et al. 2006, Van Wyk et al. 2007).

The fact that *H. mangiferae* has become associated with three cryptic species of *Ceratocystis* is not surprising. Species in this group of fungi easily establish relationships with insects (Kile 1993, Roux & Wingfield 2009), probably facilitated by the fruity aromas that they produce. The association between *C. mangicola*, *C. mangivora,* and *C. manginecans* and *H. mangiferae* is very similar to emerging new associations between ambrosia beetles and tree pathogens such as those found in Laurel Wilt Disease in the USA (Mayfield et al. 2008

In this study we chose to provide names to species reflected by phylogenetic lineages (*C. mangicola, C. mangivora,* and *C. manginecans*) rather than to treat them as population components of the single species *C. fimbriata*. In doing so we could easily define distinctly different phylogenetic groupings and provide a mechanism to distinguish their differences. Defined clades showing such differences are undoubtedly valuable in studying important diseases, including aspects of host pathogen interaction and resistance. Furthermore, providing separate names for *C. mangicola, C. mangivora,* and *C. manginecans* facilitates quarantine procedures and efforts to curb the global movement of tree pathogens (Wingfield et al. 2001, Slippers et al. 2005).

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The genus *Cladonia* (lichenized *Ascomycota, Cladoniaceae*) in South Korea

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Abstract — During a comprehensive study of *Cladonia* from South Korea, 41 taxa were recognized, including *C. dehiscens*, *C. floerkeana*, *C. macroptera*, and *C. rappii* var. *exilior*, new to South Korea. Brief information and discussion are provided for each taxon, together with a key to the taxa recorded from South Korea.

Key words — lichen-forming fungus, taxonomy, new records

Introduction

Cladonia is one of most common lichen genera, widely distributed on all continents and including more than 400 species worldwide (Ahti 2000). Park (1990) was the first to conduct an expert study of lichens in South Korea. Her 'Macrolichen flora of South Korea,' however, included only 22 *Cladonia* species, and the omission of many species we have since collected has made identification difficult. Thus, we began comprehensive research on the genus in South Korea with extensive field surveys from 2003 to 2010 and found several *Cladonia* species previously not known in this country. Here we provide information for 41 *Cladonia* taxa and a key to the South Korean species. Here we follow the taxonomic system set forth by Ahti (2000), separating South Korean *Cladonia* into five sections based on the secondary metabolites as identified by High Performance Liquid Chromatography (HPLC): *Cladonia*, *Cocciferae, Helopodium*, *Perviae,* and *Unciales. Cladina* is treated as a subgenus of *Cladonia*.

Material and methods

We examined 496 *Cladonia* specimens collected during 2003–2010 in South Korea, and deposited in the Korean Lichen Research Institute (KoLRI), Sunchon National University. External morphological descriptions were based on air-dried material, and

all specimens were observed under a Nikon SMZ645 stereomicroscope. The chemical characters were determined by color reaction according to Yoshimura (1974): K (10% aqueous KOH solution), C (saturated aqueous NaOCl solution), KC (10% KOH followed by C solution), P (5% alcoholic solution of para-phenylenediamine). Chemical compounds were identified using Thin Layer Chromatography (Culberson 1972; Elix et al. 1987; Orange et al. 2001; White & James 1985).

Taxonomy

Key to the sections of *Cladonia*

1. Podetia ecorticate, surface dull and webby ..... A. Subg. ***Cladina***
1. Podetia corticate 2
2. Apothecia and pycnidia red ..... B. Sect. ***Cocciferae***
2. Apothecia and pycnidia brown 3
3. Podetia yellowish and smooth, containing usnic acid ..... C. Sect. ***Unciales***
3. Podetia green, gray or brown, not yellowish, without usnic acid (except in *C. bacilliformis*) 4
4. Basal squamules well developed and large, podetia small or sometimes evanescent, without cups ..... D. Sect. ***Helopodium***
4. Basal squamules usually persistent, podetia usually with well developed cups, axils and cup center closed ..... F. Sect. ***Cladonia***
4. Basal squamules evanescent, podetia branched, without cups, axils open and the cup center perforate ..... E. Sect. ***Perviae***

Key to the *Cladonia* taxa recorded from South Korea

A. *Cladonia* subg. *Cladina*

1. Podetia gray, KC–, lacking usnic acid 2
1. Podetia yellowish, KC+ yellow, containing usnic acid ..... 2. ***C. arbuscula*** **subsp.** ***beringiana***
2. Ultimate branches pointing in one direction, polychotomously branched, final branchlets 2–4 ..... 32. ***C. rangiferina*** **subsp.** ***rangiferina***
2. Podetia small and slim, dichotomously branched, final branchlets 2 ..... 33. ***C. rangiferina*** **subsp.** ***grisea***

B. *Cladonia* sect. *Cocciferae*

1. Podetia usually topped with cups 2
1. Podetia without cups 5
2. Podetia sorediate ..... 29. ***C. pleurota***
2. Podetia esorediate, with granules or squamules 3
3. Podetia usually covered with granules, containing squamatic acid .. 19. ***C. granulans***
3. Podetia covered with squamules or scaly plates 4

4. Podetia covered with squamules, containing didymic acid. . . . .24. ***C. metacorallifera***
4. Podetia covered with scaly plates, containing zeorin. . . . . 6. ***C. coccifera***
5. Podetia distinctly corticate, sorediate. . . . . .14. ***C. floerkeana***
5. Podetia mostly ecorticate 6
6. Farinose soredia throughout the podetia, corticate only at the basal parts, usually without squamules . . . . . 22. ***C. macilenta***
6. Podetia entirely decorticated, granulose soredia mixed with squamules . . . . . 11. ***C. didyma***

C. *Cladonia* sect. *Unciales*

Podetia bifurcating or forming small cups at apices, containing barbatic acid . . . . . 1. ***C. amaurocraea***
Podetia pointed and richly branched, containing squamatic acid . . . . . 41. ***C. uncialis***

D. *Cladonia* sect. *Helopodium*

1. Primary thallus persist, podetia rare 2
1. Podetia usually present, primary thallus small or evanescent 3
2. Primary thallus grayish green, rather large and thick, K+ yellow . . . . . .39. ***C. symphycarpia***
2. Primary thallus yellowish green, small, K– . . . . . .4. ***C. caespiticia***
3. Podetia with squamules, P+ yellow, containing psoromic acid . . . . . .10. ***C. dehiscens***
3. Podetia verruculose on surface, P+ red, containing fumarprotocetraric acid . . . . . 27. ***C. peziziformis***

E. *Cladonia* sect. *Perviae*

1. Podetia coarsely sorediate on the tips . . . . . .36. ***C. scabriuscula***
1. Podetia esorediate 2
2. Podetia K+ yellow, containing atranorin, primary thallus abundant 40. ***C. turgida***
2. Podetia K–, primary thallus evanescent. 3
3. Podetia P+ red, containing fumarprotocetraric acid 4
3. Podetia P–, containing squamatic acid. 6
4. Podetia up to 10 cm long, with abundant squamules . . . . . .23. ***C. macroptera***
4. Podetia shorter and slim, with few squamules 5
5. Podetia simply branched, usually without apothecia. . . . . 15. ***C. furcata* var. *furcata***
5. Podetia with cymose branches, usually topped with apothecia . . . . . .16. ***C. furcata* var. *racemosa***
6. Funnel-like cups present, with large perforation in the center, podetial surface smooth and lacking squamules. . . . . 9. ***C. crispata***
6. Squamules abundant on the podetia, narrow cups present or not. . . 37. ***C. squamosa***

F. *Cladonia* sect. *Cladonia*

1. Podetia yellowish green, containing usnic acid . . . . . .3. ***C. bacilliformis***
1. Podetia not yellowish, without usnic acid 2

2. Podetia esorediate 3
2. Podetia sorediate 12

3. Podetia usually topped with cups 4
3. Podetia without cups or with shallow cups 9

4. Podetia proliferating from the center of the cups ..... 34. *C. rappii* var. *exilior*
4. Proliferation absent or on the margin of the cups 5

5. Podetia with distinct deep cups 6
5. Cups shallow, corticate inside of the cups 8

6. Podetia P+ yellow, containing psoromic acid ..... 38. *C. subconistea*
6. Podetia P+ red-brown, containing fumarprotocetraric acid 7

7. Cups small, K+ yellow, containing atranorin ..... 21. *C. kurokawae*
7. Cups large, goblet shaped, scaly plated inside of the cups, K–, without atranorin ..... 30. *C. pyxidata*

8. Cups regular, apothecia large, base of the podetia not blackened ..... 18. *C. gracilis* subsp. *turbinata*
8. Cups irregular, base of the podetia blackened, with white areolae ... 28. *C. phyllophora*

9. Podetia with smooth and continuous cortex, with areolae 10
9. Podetia lacking continuous cortex, largely decorticated 11

10. Podetia with abundant squamules, usually pointed at apices ..... 12. *C. fenestralis*
10. Podetia with few squamules, topped with small shallow cups ..... 17. *C. gracilis* subsp. *gracilis*

11. Podetia pointed at apices, rarely branched, with few squamules, sometimes small and granule-like ..... 32. *C. ramulosa*
11. Podetia with many squamules on the lower parts, small cups present on the tips, apothecia large ..... 26. *C. mongolica*

12. Podetia pointed or with tiny cups at apices 13
12. Podetia with obvious cups 15

13. Podetia corticate only at the base, with farinose soredia throughout ..... 7. *C. coniocraea*
13. Podetia corticate, with patches of soralia 14

14. Primary thallus esorediate, podetia up to 4 cm tall, with soralia on upper parts of the podetia ..... 8. *C. cornuta* subsp. *cornuta*
14. Primary thallus sorediate, podetia short, with patches of farinose soredia ..... 26. *C. ochrochlora*

15. Cups shallow, margin irregular and asymmetrical ..... 34. *C. rei*
15. Cups deep, margin entire and even 16

16. Soredia granulose, cups large and broad ..... 5. *C. chlorophaea*
16. Soredia farinose 17

17. Podetia short, cups small, K+ yellow, containing atranorin ..... 20. *C. humilis*
17. Podetia covered with farinose soredia, K–, without atranorin ..... 13. *C. fimbriata*

FIGURE 1: A. *Cladonia dehiscens*; B. *C. floerkeana*; C. *C. macroptera*; D. *C. rappii* var. *exilior*. Scale bars: A, B, D = 1 cm; C = 2 cm.

1. *Cladonia amaurocraea* (Flörke) Schaer.

REMARKS — This species is characterized by its yellowish podetia, frequently tipped with small cups. *Cladonia uncialis* resembles this species, but it does not have cups, and it contains squamatic acid but not barbatic acid. *C. arbuscula* is also yellowish with usnic acid, but its podetia lack a cortex.

This species is reported from Japan, North America and Europe (Thomson 1984; Yoshimura 1974). In South Korea, common, usually on rock, mostly found in the northern part and over the altitude of 800 m.

REPRESENTATIVE SPECIMENS (OF 15) EXAMINED — **SOUTH KOREA. GANGWON PROV.**, Taebaek city, Mt. Naejang, 35°29′47.2″N 126°53′44.5″E, alt. 1080 m, Hur 030419; Mt. Sorak, 38°07′14.5″N 128°23′03.5″E, alt. 1355 m, Hur 041490; **GYEONGBUK PROV.**, Mungyeong city, Mt. Juhul, 36°46′58.5″N 128°05′21.3″E, Hur 040160; **GYEONGNAM PROV.**, Mt. Gaya, 35°49′11.3″N 128°07′18.2″E, alt. 1440 m, Hur 040215.

2. *Cladonia arbuscula* subsp. ***beringiana*** Ahti

REMARKS — The subspecies is resembles *C. rangiferina* but with yellowish podetia due to the presence of usnic acid. A worldwide complex with many subspecies and chemotypes, *C. arbuscula* is usually represented by *C. arbuscula* subsp. *beringiana* (Ahti 2000) in eastern Asia and (in Japan) subsp. *mitis* (Yoshimura 1974, Harada et al. 2004).

This subspecies is reported from North America, Europe, Japan and China (Harada et al. 2004; Thomson 1984; Wei 1991). In South Korea, only one specimen was found on soil during our study.

Specimen examined — **SOUTH KOREA. Gyeongbuk Prov.**, Mt. Juwang, 36°23′47.7″N 129°08′50.5″E, alt. 270 m, Hur 050584.

3. ***Cladonia bacilliformis*** (Nyl.) Vain.

Remarks — Characterized by having soredia all over the podetia, this species might be confused with the sorediate species *C. ochrochlora* and *C. coniocraea* but differs in having yellowish podetia and containing usnic acid as the main compound.

Cladonia bacilliformis is also known from North America and China (Thomson 1984; Wei 1991). In South Korea, very rare, found only in some low altitude mountains growing on soil or rock.

Specimens examined — **SOUTH KOREA. Jeonnam Prov.**, Mt. Ilrim, 34°40′43.2″N 127°00′41.8″E, alt. 360 m, Hur 050375. Mt. Cheongwan, 34°32′39.8″N 126°56′51.5″E, alt. 200 m, Hur 050527. **Gyeonggi Prov.**, Mt. Gakhul, 38°06′55.1″ N 127°22′49.6″ E, alt. alt. 826 m, Hur 080336.

4. ***Cladonia caespiticia*** (Pers.) Flörke

Remarks — Diagnosed by very short (≤ 0.5 cm) podetia and pycnidia on the primary thallus, *C. caespiticia* might be confused with *C. symphycarpia* but lacks norstictic acid and has a smaller thinner primary thallus.

Also known from North America, Europe, South Africa, and Asia (Park 1990); not common or widespread in South Korea, where it grows on soil or rock at altitudes below 900 m.

Specimens examined — **SOUTH KOREA. Jeonnam Prov.**, Mt. Baekun, 35°04′17.4″N 127°39′27.1″E, alt. 840 m, Hur 041268. **Gangwon Prov.**, Mt. Sorak, 38°10′46.7″N 128°21′54.7″E, alt. 430 m, Hur 041536. **Chungnam Prov.**, Mt. Gaeryeong, 36°21′30.7″N 127°12′44.5″E, alt. 750 m, Hur 041620.

5. ***Cladonia chlorophaea*** (Flörke ex Sommerf.) Spreng.

Remarks — Characterized by wide-open cups and granulose soredia, *C. chlorphaea* could be confused with *C. grayi,* which differs in producing grayanic acid, and *C. pyxidata*, which has granular squamules but no soredia.

Widely distributed from the temperate zone to the Antarctic/Arctic (Ahti 2000), *C. chlorophaea* is rather common in South Korea, where it grows on exposed soil (or sometimes rock or moss) at altitudes above 1000 m.

Representative specimens (of 13) examined — **SOUTH KOREA. Jeju Prov.**, Mt. Halla, 33°05′48.8″N 126°55′11.6″E, alt. 1325 m, Hur 030371. **Gangwon Prov.**, Mt. Hambaek, 37°11′48.6″N 128°55′06.5″E, alt. 1194 m, Hur 030689. Mt. Odae, 37°47′55.3″N 128°32′53.3″E, alt. 1490 m, Hur 040472. **Jeonbuk Prov.**, Mt. Palgong, 35°36′01.8″N 127°27′57.4″E, alt. 688 m, Hur 050400.

6. *Cladonia coccifera* (L.) Willd.

Remarks — Characterized by large, red apothecia on the proliferation, surface squamules, and usnic acid and zeorin as the secondary metabolites, *C. coccifera* resembles *C. pleurota* but lacks soredia while *C. pleurota* has granulose soredia.

Circumpolar in the northern hemisphere south to the Himalayas (Stenroos 1989), *C. coccifera* is uncommon in South Korea where it is found on rock or soil at 15–1000 m or above.

Specimens examined — **SOUTH KOREA. Jeonnam Prov.**, Gohong county, Sorok Island, 35°48′11.2″N 129°18′47.3″E, alt. 15 m, Hur 030063-1. **Gangwon Prov.**, Mt. Gariwang, 37°24′05.0″N 128°32′39.5″E, alt. 937 m, Hur 080058, Hur 080065. Mt. Tuda, 37°26′36.8″N 128°59′35.3″E, alt. 761 m, Hur 080110. **Gyeongbuk Prov.**, Mt. Joryeong, 37°48′27″N 128°03′32″E, alt. 500 m, Hur 080303.

7. *Cladonia coniocraea* (Flörke) Spreng.

Remarks — This species may be confused with *C. ochrochlora* but has extensively spreading farinose soredia over podetia rather than round soralia. It is mostly decorticated, while *C. ochrochlora* has a well-developed cortex all over the podetia.

Known from the northern hemisphere and Australia (Park 1990), *C. conciocraea* is uncommon in South Korea where it is found on rock or moss.

Specimens examined — **SOUTH KOREA. Gangwon Prov.**, Mt. Naejang, 35°29′47.2″N 126°53′44.5″E, alt. 1080 m, Hur 030423. Mt. Sorak, 38°06′40.4″N 128°24′26.8″E, alt. 1335 m, Hur 041452. **Jeju Prov.**, Jeju Island, 33°24′26.6″N 126°29′46.0″E, alt. 600 m, Hur 040634-1.

8. *Cladonia cornuta* (L.) Hoffm. subsp. ***cornuta***

Remarks — Very close to *C. coniocraea*, which is corticate only at the base, this subspecies has a more extensive cortex and patches of soralia. *Cladonia ochrochlora* is also similar, but it is short and stout, its podetia are usually topped with small cups, and the primary thallus has soredia, while *C. cornuta* subsp. *cornuta* lacks cups, is slim and tall, and has an esorediate primary thallus.

Circumpolar, boreal, arctic, and in the southern hemisphere (Thomson 1984), the subspecies is rather common throughout South Korea, usually growing on soil, sometimes on wood or rock.

Representative specimens (of 22) examined — **SOUTH KOREA. Jeonnam Prov.**, Mt. Jiri, 35°18′39.4″N 127°35′54.3″E, alt. 1620 m, Hur 040294. **Jeju Prov.**, Jeju Island, 33°21′30.4″N 126°31′19.3″E, alt. 1710 m, Hur 040740. **Jeonbuk Prov.**, Mt. Naejang, 35°29′46.1″N 126°53′56.7″E, alt. 600 m, Hur 050021. **Gangwon Prov.**, Mt. Sorak, 38°08′53.5″N 128°20′05.3″E, alt. 1080 m, Hur 050279.

9. *Cladoonia crispata* (Ach.) Flot.

Remarks — The funnel-like cups make this species quite unique. *Cladonia crispata* occurs on soil and may be confused with *C. furcata*, which, however,

does not form cups and the tips and contains fumarprotocetraric acid but not squamatic acid.

Circumpolar, arctic, temperate, it is also found in South America (Thomson 1984), *C. crispata* is very rare in South Korea, where it usually grows on soil or thin soil over rock at altitudes c. 1000 m.

Specimens examined — **SOUTH KOREA. Jeonnam Prov.**, Mt. Baekun, 35°10′ N 127°60′ E, alt. 1045 m, Hur 030291. **Gangwon Prov.**, Mt. Taebaek, 37°06′15.5″ N 128°56′06.2″ E, alt. 1000 m, Hur 030391, Hur 030394.

10. ***Cladonia dehiscens*** Vain. Fig. 1A

Remarks — New to South Korea, *C. dehiscens* may be confused with *C. peziziformis*, but it contains psoromic acid only, the podetia surfaces are partly decorticated and squamulose; *C. peziziformis* contains atranorin, and the surface is verruculose without squamules.

Previously known only from China (Wei 1991), the species is rather rare in South Korea, where it was found only found at c. 250 m on Mt. Gumsan (Gyeongnam province), growing on rock.

Specimens examined — **SOUTH KOREA. Gyeongnam Prov.**, Namhae county, Mt. Gumsan, 34°45′38.5″N 128°02′55.5″E, alt. 249 m, Hur 070944, Hur 070945.

11. *Cladonia didyma* (Fée) Vain.

Remarks — In having red apothecia and soredia, *C. didyma* is resembles *C. macilenta*. The latter species, however, has thamnolic acid as the main chemical compound and usually farinose soredia all over the podetia, while *C. didyma* has didymic acid and the granulose soredia mix with squamules.

Pantropical, extending to warm temperate areas (Ahti 2000). In South Korea, only one specimen was found on bark from the Jeju Island.

Specimen examined — **SOUTH KOREA. Jeju Prov.**, Jeju Island, 33°26′04.4″N 126°34′01.7″E, alt. 545 m, Hur 040878.

12. ***Cladonia fenestralis*** Nuno

Remarks — This species might be confused with *C. gracilis*, but *C. fenestralis* has many more squamules on the podetia (which are usually taller) and inconspicuous cups. *Cladonia gracilis* has regular shallow cups on the tips of the podetia.

This species has been reported from Southeast Asia (Sipman 1993) and South Korea (Hur et al. 2005). During our study, only two specimens were found growing on rocks from Jeonbuk and Gangwon provinces.

Specimens examined — **SOUTH KOREA. Jeonbuk Prov.**, Mt. Seonun, 35°29′46.9″N 126°53′40.7″E, alt. 300 m, Hur 030240. **Gangwon Prov.**, Mt. Naejang, 35°29′47.2″N 126°53′44.5″E, alt. 1080 m, Hur 030421.

13. *Cladonia fimbriata* (L.) Fr.

Remarks — Characterized by slim podetia, narrow cups, and farinose soredia, *C. fimbriata* is closely resembles *C. chlorophaea*, which differs in wider cups and granulose soredia.

A common temperate to boreal and arctic-antarctic species (Ahti 2000), *C. fimbriata* is very rare in South Korea, found only in Chungbuk province, growing on rock.

Specimen examined — **SOUTH KOREA. Chungbuk Prov.**, Mt. Sobaek, 36°57′18″N 128°28′52.3″E, alt. 1389 m, Hur 030769.

14. *Cladonia floerkeana* (Fr.) Flörke Fig. 1B

Remarks — New to South Korea, *C. floerkeana* might be confused with another sorediate species *C. macilenta*, but the latter species has farinose soredia and the whole podetia are almost entirely decorticated, while *C. floerkeana* is corticate over most of the podetia, its soredia are granulose, and it has squamules on the podetia.

Also reported from North America, Europe, Australasia (Ahti 2000), and East Asia (Wei 1991), *C. floerkeana* is very rare in South Korea, where it was found growing on bark at c. 300 m on Mt. Juwang.

Specimen examined — **SOUTH KOREA. Gyeongbuk Prov.**, Mt. Juwang, 36°23′47.7″N 129°08′50.5″E, alt. 270 m, Hur 050587.

15. *Cladonia furcata* (Huds.) Schrad. var. ***furcata***

Remarks — This variety is easily recognizable by having a well-developed cortex, simple branches, and a few squamules. It has many variations and forms; some Korean specimens, which are rather long and stout (≤ 8 × 2 mm), would have been referred to *C. furcata* var. *pinnata* (Fink 1904), but this has recently been synonymised with *C. furcata* var. *furcata* (Hur et al. 2005).

A widespread warm to cold temperate species (Ahti 2000), *C. furcata* is the most common species in South Korea, widely distributed from low to high altitudes, usually growing on humus or soil over rock, under the shady forest.

Representative specimens (of 53) examined — **SOUTH KOREA. Jeonnam Prov.**, Mt. Baekun, 35°10′ N 127°60′ E, alt. 1050 m, Hur 030289. **Gangwon Prov.**, Mt. Taebaek, 37°06′25.9″N 128°56′47.8″E, alt. 963 m, Hur 030633. **Chungbuk Prov.**, Mt. Sobaek, 36°57′17.6″N 128°28′47.1″E, alt. 1394 m, Hur 030778. **Gyeongbuk Prov.**, Mt. Juhul, 36°46′58.5″N 128°05′21.3″E, alt. 675 m, Hur 040157. **Chungnam Prov.**, Mt. Gaeryong, 36°21′25.6″N 127°12′35.3″E, alt. 770 m, Hur 041628.

16. *C. furcata* var. ***racemosa*** (Hoffm.) Flörke

Remarks — One additional variety of *C. furcata* has been found in Korean specimens, which differs from other species by the subradiate branching

towards the apex, the microsquamules on the surface, and the apothecia commonly present on the cymose branch tips. Thallus P + red, containing fumarprotocetraric acid.

Specimen examined — **SOUTH KOREA. Gangwon Prov.**, Mt. Odae, 37°47′43.3″N 128°34′22.0″E, alt. 1240 m, Hur 040481.

17. *Cladonia gracilis* (L.) Willd. subsp. ***gracilis***

Remarks — Similar to *C. furcata*, *C. gracilis* subsp. *gracilis* is much more slender and has fewer squamules and podetia tips usually topped with cups. The similar *C. maxima* lacks a primary thallus and has much longer, unbranched podetia. The subspecies also differs from *C. cornuta* by lacking soredia.

Distributed from circumpolar regions to South America (Thomson 1984), this subspecies is rare in South Korea, usually found growing on rock at altitudes above 900 m.

Specimens examined — **SOUTH KOREA. Jeonbuk Prov.**, Mt. Naejang, 35°29′46.1″N 126°53′56.7″E, alt. 600 m, Hur 050020. **Jeju Prov.**, Mt. Halla, 33°21′53.8″N 126°30′40.3″E, alt. 1695 m, Hur 090207. **Gangwon Prov.**, Mt. Jobong, 37°56′10.7″N 128°33′74.7″E, alt. 980 m, Hur 090256.

18. *Cladonia gracilis* subsp. ***turbinata*** (Ach.) Ahti

Remarks — This subspecies differs from subsp. *gracilis* mainly by having large, wide cups and being covered with squamules. The similar *C. rappii* differs in proliferating from the center of the podetial cups. The also similar *C. phyllophora* differs in having regular cups and a smooth cortex.

Cosmopolitan (Ahti 1980), *C. gracilis* subsp. *turbinata* is very common and widespread in South Korea on soil or mosses over rock at altitudes above 1000 m.

Representative specimens (of 24) examined — **SOUTH KOREA. Jeju Prov.**, Mt. Halla, 33°05′48.8″N 126°55′11.6″E, alt. 1655 m, Hur 030361; alt. 800 m, Hur 030368. **Jeonnam Prov.**, Mt. Jiri, 35°19′09.4″N 127°41′15.9″E, alt. 1600 m, Hur 040348. **Gangwon Prov.**, Mt. Taebaek, 37°06′10.4″N 128°57′16.1″ E, alt. 1120 m, Hur 041021.

19. *Cladonia granulans* Vain.

Remarks — Characterized by slender podetia covered by squamules or granules on the podetia, *C. granulans* is quite similar to *C. metacorallifera* but lacks didymic acid and has no proliferation on the cup margins.

Originally described from Japan and also known from North America (Thomson 1984), this species is rare in South Korea and usually found at altitudes above 1000 m, growing on bark or soil.

Specimens examined — **SOUTH KOREA. Gangwon Prov.**, Mt. Naejang, 35°29′47.2″N 126°53′44.5″E, alt. 1050 m, Hur 030418. **Jeju Prov.**, Jeju Island, 33°21′42.4″N 126°32′56.9″E, alt. 1670 m, Hur 040819. **Chungbuk Prov.**, Mt. Sobaek, 36°55′47.5″ N 128°27′22.9″ E, alt. 1301 m, Hur 070338.

20. *Cladonia humilis* (With.) J.R. Laundon

Remarks — *Cladonia humilis,* which contains atranorin, is difficult to differentiate from *C. chlorophaea*, which lacks atranorin and has larger podetia.

A widespread temperate species (Ahti 2000), *C. humilis* is uncommon in South Korea, where it is found on soil over rock at low altitudes.

Specimens examined — **SOUTH KOREA. Jeonnam Prov.**, Sorok Island, 35°48′11.2″N 129°18′47.3″E, alt. 60 m, Hur 030056. **Gyeongbuk Prov.**, Mt. Juhul, 36°46′58.5″N 128°05′21.3″E, alt. 657 m, Hur 040156; 36°46′46.0″N 128°06′02.1″E, alt. 845 m, Hur 040151. **Chungbuk Prov.**, Mt. Joryeong, 37°01′33.3″N 128°11′59.2″E, alt. 285 m, Hur 061110.

21. *Cladonia kurokawae* Ahti & S. Stenroos

Remarks — Characterized by small granules rather than real soredia, somewhat small podetia and cups, fumarprotocetraric acid, and atranorin, *C. kurokawae* resembles *C. subconistea*, which lacks fumarprotocetraric acid and contains psoromic acid as the main compound.

Originally described from East Asia (Lai 2000), *C. kurokawae* is rather common in South Korea where it is found mostly on soil or mossy rock in low altitude mountains.

Representative specimens (of 17) examined — **SOUTH KOREA. Jeonnam Prov.**, Mt. Baekun, 36°57′14.5″N 128°29′18.8″E, alt. 1000 m, Hur 0300182-2. **Jeonbuk Prov.**, Mt. Naejang, 35°48′11.2″N 126°18′47.3″E, alt. 600 m, Hur 030453. **Gyeongnam Prov.**, Mt. Gumsan, 34°45′44.2″N 127°59′29.6″E, alt. 290 m, Hur 040031. **Gyeonggi Prov.**, Mt. Myeongji, 37°55′57.7″N 127°28′53.3″E, alt. 223 m, Hur 080669.

22. *Cladonia macilenta* Hoffm.

Remarks — This species is characterized by having red apothecia and farinose soredia all over the podetia. TLC detected two chemical races in Korean materials: (1) didymic and thamnolic acids; (2) didymic, barbatic, and usnic acids. The horizontal primary thallus generally contains only thamnolic acid (Asahina 1970).

Widespread worldwide (Ahti 2000), *C. macilenta* is also common and widespread in South Korea, where it usually grows on rotting wood, bark, humus, or soil at altitudes above 500 m.

Representative specimens (of 14) examined — **SOUTH KOREA. Gangwon Prov.**, Mt. Hambaek, 37°11′48.6″N 128°55′06.5″E, alt. 1194 m, Hur 030685. Mt. Seokbyeong, 37°34′29.9″N 128°51′21.8″E, alt. 686 m, Hur 080188. **Jeju Prov.**, Jeju Island, 33°24′26.6″N 126°29′46.0″E, alt. 600 m, Hur 040634. **Jeonnam Prov.**, Wando Island, 34°21′10.3″N 126°41′10.9″E, alt. 535 m, Hur 050142.

23. *Cladonia macroptera* Räsänen Fig. 1C

Remarks — Characterized by long (≤ 10 cm) podetia, *C. macroptera* differs from *C. furcata* by tall thick podetia with rare branches and densely squamulose

and an areolate cortex that differs from the more or less continuous cortex in *C. furcata.*

Previously known only from China and Canada (Brodo et al. 1987, Wei 1991), *C. macroptera* is new to South Korea where two specimens were found growing on moss over rock.

Specimens examined — **SOUTH KOREA. Jeonnam Prov.**, Mt. Duryon, 37°06′27.6″N 128°56′48.8″E, alt. 200 m, Hur 030314. **Gangwon Prov.**, Mt. Odae, 37°47′36.2″N 128°34′37.0″E, alt. 1195 m, Hur 040483.

24. *Cladonia metacorallifera* Asahina

Remarks — This species is characterized by having cups topped with red apothecia, without soredia but squamulose and contains squamatic acid. It may be confused with *C. coccifera*, but the latter species usually has farinose granules resembling soredia inside of the cups and contains zeorin.

Also known from North America, Japan, and China (Thomson 1984; Wei 1991), *C. metacorallifera* is uncommon in South Korea where it usually grows on soil over rock at 1000–1700 m altitudes.

Specimens examined — **SOUTH KOREA. Gangwon Prov.**, Mt. Naejang, 35°29′47.2″N 126°53′44.5″E, alt. 1080 m, Hur 030420. Mt. Sorak, 38°06′42.8″N 128°24′21.8″E, alt. 1360 m, Hur 041466. **Jeju Prov.**, Jeju Island, 33°21′32.9″N 126°31′54.9″E, alt. 1920 m, Hur 040758. **Jeonnam Prov.**, Mt. Jiri, 35°19′40.9″N 127°44′15.0″E, alt. 1564 m, Hur 060686; 35°18′59.5″N 127°42′26.1″E, alt. 1659 m, Hur 060955.

25. *Cladonia mongolica* Ahti

Remarks — Characterized by dense squamules on the lower podetia and usually with large apothecia, *C. mongolica* resembles *C. scabriuscula* but lacks soredia near the podetia tips.

Also reported from China (Wei 1991), the species is rather rare in South Korea and grows on soil or rock. Kwang-Hee Moon reported *C. mongolica* from Mt. Sorak (Moon 1999), while we have found more specimens from different localities in the country.

Specimens examined — **SOUTH KOREA. Jeonnam Prov.**, Mt. Duryon, 37°06′27.6″N 128°56′48.8″E, alt. 200 m, Hur 030318. **Gangwon Prov.**, Mt. Hambaek, 37°11′48.6″N 128°55′06.5″E, alt. 1194 m, Hur 030686. **Chungbuk Prov.**, Mt. Sobaek, 36°57′27.0″N 128°26′40.7″E, alt. 618 m, Hur 030711.

26. *Cladonia ochrochlora* Flörke

Remarks — Characterized by farinose soredia, this species might be confused with *C. coniocraea*, which differs by being sorediate all over the podetia while *C. ochrochlora* has continuous cortex. It is also similar to the yellowish *C. bacilliformis* but lacks usnic acid so the thallus color is greener.

Mainly distributed in temperate and boreal areas (Ahti 2000), *C. ochrochlora* is not very common in South Korea, where it occurs mostly in the northern part at altitudes above 1000 m.

Specimens examined — **SOUTH KOREA. Jeonnam Prov.**, Mt. Jiri, 35°18′07.4″N 127°34′00.1″E, alt. 1440 m, Hur 060266. **Jeonbuk Prov.**, Mt. Deogyu, 35°51′05.3″N 127°44′55.4″E, alt. 1576 m, Hur 060506. **Gangwon Prov.**, Mt. Taebaek, 37°05′46.1″N 128°55′05.6″E, alt. 1572 m, Hur 070633. Mt. Gariwang, 37°24′05.0″N 128°32′39.5″E, alt. 937 m, Hur 080026, Hur 080029.

27. *Cladonia peziziformis* (With.) J.R. Laundon

Remarks — Reported previously from South Korea (Wang et al. 2008), *C. peziziformis* is characterized by short verruculose podetia and roundish primary squamules. It is very similar to *C. cariosa,* which, however, contains atranorin. It also resembles *C. subcariosa*, which has significantly elongated primary squamules.

Primarily a temperate species that prefers mineral soil, *C. peziziformis* is common in the eastern United States, Europe, Japan, and China (Ahti 2000). In South Korea, only one specimen from Jeonnam province was found, growing on soil.

Specimen examined — **SOUTH KOREA. Jeonnam Prov.**, Mt. Baeka, 35°09′08.3″N 127°09′45.2″E, alt. 550 m, Bae 080032.

28. *Cladonia phyllophora* Ehrh. ex Hoffm.

Remarks — Characterized by whitish spots near the basal parts and quite similar to *C. gracilis* subsp. *turbinata, C. phyllophora* has more irregular cups, a decorticated area under the cups, and a base that usually blackens.

Distributed in circumpolar, boreal, and low arctic regions (Thomson 1984), *C. phyllophora* is common in South Korea, usually on soil over rock at altitudes above 1000 m,.

Representative specimens (of 10) examined — **SOUTH KOREA. Jeonnam Prov.**, Mt. Jiri, 35°20′05.0″N 127°42′49.4″E, alt. 1525 m, Hur 040394. **Gangwon Prov.**, Mt. Hambaek, 37°11′48.6″N 128°55′06.5″E, alt. 1194 m, Hur 030687. Mt. Sorak, 38°09′17.4″N, E128°26′25.6″E, alt. 1255 m, Hur 050300.

29. *Cladonia pleurota* (Flörke) Schaer.

Remarks — Characterized by cups bearing granulose soredia, *C. pleurota* may be mistaken for *C. carneola* when the pycnidia are brownish but differs in having zeorin and isousnic acid.

Circumpolar in the arctic and the boreal forest (Thomson 1984), *C. pleurota* is not common in South Korea and is restricted to the northeastern part, usually on rock, soil, and at the base of the trees.

Specimens examined — **SOUTH KOREA. Gangwon Prov.**, Mt. Gariwang, 37°24′05.0″N 128°32′39.5″E, alt. 937 m, Hur 080057. Mt. Taebaek, 37°12′35.7″N 128°55′13.0″E, alt. 1400 m, Hur 080297. **Gyeongbuk Prov.**, Mt. Joryeong 37°48′27″N 128°03′32″E, alt. 500 m, Hur 080304.

30. *Cladonia pyxidata* (L.) Hoffm.

Remarks — Characterized by bell-shaped cups covered inside and out by round squamules, *C. pyxidata* it may be confused with *C. chlorophaea*, which differs in usually wider and goblet shaped cups (that are sometimes narrow when young) that have granulose soredia inside the cups.

This is a cosmopolitan species (Thomson 1984). In South Korea, it is not widespread, growing on rock or soil, at the elevation lower than 1000 m.

Specimens examined — **SOUTH KOREA. Chungbuk Prov.**, Mt. Worak, 36°51′36.6″N 128°05′27.4″E, alt. 245 m, Hur 041158. **Gangwon Prov.**, Mt. Sorak, 38°08′53.5″N 128°20′05.3″E, alt. 1000 m, Hur 050275. **Jeonnam Prov.**, Mt. Dalma, 34°22′45.2″N 126°35′11.6″E, alt. 456 m, Hur 050344.

31. *Cladonia ramulosa* (With.) J.R. Laundon

Remarks — The species is characterized by squamules on the lower part of the podetia and partly decorticated. Morphologically, *C. rei* is very similar, but *C. ramulosa* lacks powdery soredia on the upper podetia and has translucent decorticated areas on the podetia. *Cladonia scabriuscula,* which might be confused with *C. ramulosa,* lacks homosekikaic acid.

A widespread temperate to tropical species (Ahti 2000), it is rather common in South Korea, usually on soil or rock and found at altitudes from sea level to above 1000 m.

Representative specimens (of 26) examined — **SOUTH KOREA. Jeonnam Prov.**, Sorok Island, 35°48′11.2″N 129°18′47.3″E, alt. 60 m, Hur 030058. **Gangwon Prov.**, Mt. Taebaek, 37°06′15.5″N 128°56′06.2″E, alt. 1000 m, Hur 030392. Mt. Odae, 37°47′43.3″N 128°34′22.0″E, alt. 1240 m, Hur 040480. **Jeju Prov.**, Jeju Island, 33°34′00.1″N 126°45′44.4″E, alt. 10 m, Hur 090031.

32. *Cladonia rangiferina* (L.) Weber ex F.H. Wigg. subsp. ***rangiferina***

Remarks — The subspecies is easily recognizable by having grayish and webby podetial surface, with branches curved and facing a same direction. It is very similar to *C. rangiferina* subsp. *grisea,* but the branchlets of the latter subspecies are mainly isotomic dichotomously branched, and the podetium is thinner and smaller.

Circumpolar arctic to temperate in distribution (Thomson 1984), it is rather common over rock or soil in South Korea at altitudes above 1000 m.

Representative specimens (of 30) examined — **SOUTH KOREA. Jeju Prov.**, Mt. Halla, 33°57′32.6″N 126°29′17.5″E, alt. 1980 m, Hur 030357. **Gyeongbuk Prov.**, Cheongryangsa Temple, 36°47′25.2″N 128°55′25.4″E, alt. 885 m, Hur 040102. **Jeonnam Prov.**, Mt. Jiri, 35°19′03.9″N 127°41′22.7″E, alt. 1660 m, Hur 040352. Gangwon Prov., Mt. Gariwang, 37°24′05.0″N 128°32′39.5″E, alt. 937 m, Hur 080059.

33. *Cladonia rangiferina* subsp. *grisea* Ahti

REMARKS — See under *C. rangiferina* subsp. *rangiferina*.

The subspecies is fairly common on Japanese mountains and also known from Korea, Taiwan and China (Ahti 1961). In South Korea, common and usually growing on rock under the open canopy.

REPRESENTATIVE SPECIMENS (OF 8) EXAMINED — **SOUTH KOREA. JEONNAM PROV.**, Mt. Wolchul, 34°45′29.5″N 126°40′46.5″E, alt. 400 m, Hur 030115. **JEJU PROV.**, Jeju Island, 33°21′37.7″N 126°32′40.3″E, alt. 1755 m, Hur 040838. **GANGWON PROV.**, Mt. Sorak, 38°06′40.1″N 128°24′33.9″E, alt. 1335 m, Hur 041437. **GYEONGBUK PROV.**, Mt. Juwang, 36°23′47.7″N 129°08′50.5″E, alt. 270 m, Hur 050590.

34. *Cladonia rappii* var. ***exilior*** (Abbayes) Ahti FIG. 1 D

REMARKS — A new record for South Korea, this variety is characterized by proliferating podetia and podetia cups that are usually very small. It is similar to *C. cervicornis,* which has 4–6 proliferation tiers compared to the 2–3 tiers usual for this variety.

Cladonia rappii is distributed in North America, Asia, Africa, and Melanesia (Ahti 2000). In South Korea, only this variety is found and mainly occurs in the southwestern part of Korea, usually growing on rocks.

SPECIMENS EXAMINED — **SOUTH KOREA. CHUNGBUK PROV.**, Mt. Sobaek, 36°57′05.5″N 128°29′27.4″E, alt. 1375 m, Hur 030758. Mt. Joryeong, 36°49′00.9″N 128°02′53.7″E, alt. 784 m, Hur 061048. **JEONNAM PROV.**, Mt. Jiri, 35°15′59.2″N 127°34′40.7″E, alt. 530 m, Hur 040231; 35°19′54.5″N 127°43′04.7″E, alt. 1657 m, Hur 060701. **GYEONGNAM PROV.**, Mt. Gaya, 35°48′36.9″N 128°08′20.9″E, alt. 746 m, Hur 060103.

35. *Cladonia rei* Schaer.

REMARKS — Characterized by largely decorticated podetia with small cups and coarse soredia, *C. rei* resembles *C. gracilis* subsp. *gracilis,* except for the presence of the soredia, and homosekikaic acid. It could be confused with *C. scabriuscula,* which lacks cups on the apex and homosekikaic acid and has squamulose podetia.

This is a cosmopolitan species (Galloway 2007). In South Korea, it is rather rare and grows on soil or rock at altitudes below 1000 m.

SPECIMENS EXAMINED — **SOUTH KOREA. GANGWON PROV.**, Mt. Taebaek, 37°06′28.4″N 128°56′47.8″E, alt. 944 m, Hur 030626. **JEONNAM PROV.**, Mt. Baeka, 34°32′33.1″N 126°55′46.7″E, alt. 334 m, Hur 050556.

36. *Cladonia scabriuscula* (Delise) Leight.

REMARKS — Characterized by dichotomously branched podetia and having granulose soredia at the apex, *C. scabriuscula* may be confused with *C. squamosa*, except for the different chemical compounds and absence of squamatic acid. It also resembles *C. furcata,* which differs in having a continuous cortex and lacking soredia on the podetia.

Widespread worldwide in primarily temperate, oceanic regions (Ahti 2000), *C. scabriuscula* is rather common and widespread throughout South Korea, usually on rock or soil over rock.

Representative specimens (of 33) examined — **SOUTH KOREA. Gangwon Prov.**, Mt. Odae, 37°47′43.3″N 128°34′22.0″E, alt. 1240 m, Hur 040482. **Jeonbuk Prov.**, Mt. Deogyu, 35°52′13.5″N 127°46′42.3″E, alt. 830 m, Hur 050061. **Jeonnam Prov.**, Mt. Hulseok, 34°41′21.4″N 126°40′51.4″E, alt. 203 m, Hur 050476.

37. *Cladonia squamosa* (Scop.) Hoffm.

Remarks — This species is hard to identify when the squamules are greatly reduced and appear to be granular or even soredia; key characters are possession of squamatic acid and the lack of real soredia.

Widespread in temperate and colder regions in both northern and southern hemispheres (Ahti 2000), *C. squamosa* is common but not widespread in South Korea on soil or rock, sometimes at the base of a tree.

Representative specimens (of 9) examined — **SOUTH KOREA. Jeonnam Prov.**, Mt. Wolchul, 36°56′50.5″N 128°29′51.2″E, alt. 400 m, Hur 030137. **Chungbuk Prov.**, Mt. Sobaek, 36°57′11.2″N 128°28′37.2″E, alt. 1404 m, Hur 030792. **Gangwon Prov.**, Mt. Sorak, 38°06′43.0″N 128°24′21.3″E, alt. 1375 m, Hur 041470.

38. ***Cladonia subconistea*** Asahina

Remarks — Diagnosed by psoromic acid, *C. subconistea* resembles *C. humilis* in having small podetia and cups but lacks the soredia and fumarprotocetraric acid found in the latter.

Known only from east Asia (Harada et al. 2004, Hur et al. 2005, Wei 1991), *C. subconistea* is very rare in South Korea, where it is found growing on rock, usually at altitudes below 500 m.

Specimens examined — **SOUTH KOREA. Gangwon Prov.**, Mt. Chiak, 37°17′43.6″N 128°01′20.1″E, alt. 515 m, Hur 040562. **Gyeongnam Prov.**, Mt. Cheondae, 36°09′16.0″N 127°37′19.1″E, alt. 196 m, Hur 061145.

39. *Cladonia symphycarpia* (Flörke) Fr.

Remarks — Characterized by its short podetia (< 1 cm) and containing norstictic acid, *C. symphycarpia* (frequently misspelled as '*symphycarpa*,' see Ahti 2000) usually lacks podetia, so that it must be recognized based on the primary thallus alone.

Widespread in Eurasia and northern North America (Ahti 2000), the species is rather rare in South Korea and is restricted to the Sorak Mountains, growing on soil over rock.

Specimens examined — **SOUTH KOREA. Gangwon Prov.**, Mt. Sorak, 38°07′37.6″N 128°27′48.5″E, alt. 1300 m, Hur 041581; 38°09′37.7″N 128°19′26.5″E, alt. 660 m, Hur 050256, Hur 050260.

40. *Cladonia turgida* Hoffm.

REMARKS — The species is characterized by having atranorin, a large primary thallus, and distorted podetia with a well-developed cortex.

The species is circumpolar, arctic to north temperate (Thomson 1984). In South Korea, not widespread, usually occurs in the southern part, on soil over rock.

REPRESENTATIVE SPECIMENS (OF 11) EXAMINED — **SOUTH KOREA. JEONNAM PROV.**, Mt. Jiri, 35°15′59.2″N 127°34′40.7″E, alt. 530 m, Hur 040227; 35°19′40.9″N 127°44′15.0″E, alt. 1564 m, Hur 060687. Mt. Dalma, 34°22′45.4″N 126°35′13.6″E, alt. 445 m, Hur 050355. **JEJU PROV.**, Mt. Halla, 33°21′18.8″N 126°30′00.4″E, alt. 1492 m, Hur 080778.

41. *Cladonia uncialis* (L.) F.H. Wigg.

REMARKS — Some specimens from Japan are reported to contain hypothamnolic acid (Yoshimura 1974) and squamatic acid is reported in material from America (Thomson 1984), but neither chemotype is found in Korean specimens. *C. uncialis* is unique in having yellowish and dichotomously branched podetia and forming dense tufts. Quite similar to *C. amaurocraea*, *C. uncialis* differs in having usually smaller podetia and usnic acid and lacking cups and no barbatic acid.

The species is widespread, circumpolar, and arctic to temperate (Ahti 2000). Rare in South Korea, only one specimen has been found from Gyeongnam province, growing on soil.

SPECIMEN EXAMINED — **SOUTH KOREA. GYEONGNAM PROV.**, Mt. Gaya, 35°49′11.3″N 128°07′18.2″E, alt. 1440 m, Hur 040220.

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First record of *Resupinatus poriaeformis* (*Agaricomycetes*) from South America

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Abstract — *Resupinatus poriaeformis* is reported as new to South America. Description of the species and a key to the accepted species of the genus reported to South America are provided.

Key words — *Tricholomataceae*, diversity

Introduction

Resupinatus was validated by Gray in 1821 and comprises about 15–20 valid species (www.indexfungorum.org; http://www.cbs.knaw.nl; Kirk et al. 2008). It is a cosmopolitan genus with small, resupinate, or pendent basidiomata, a partially gelatinized context, and clamped hyphae (Singer 1962; Thorn 1986; Pegler 1986). Recently Thorn et al. (2005) placed some cyphelloid fungi or "reduced agarics" with cup-shaped basidiomata into *Resupinatus* based on molecular and morphological studies.

Although some species of *Agaricales* have been registered in mangroves, there are no reports of *Resupinatus* from this ecosystem (Hibbett & Binder 2001; Baltazar et al. 2009). However there are five reports of the genus from South America: *R. alboniger* (Pat.) Singer 1978, *R. applicatus* (Batsch) Gray 1821, *R. dealbatus* (Berk.) Singer 1973, *R. graminum* (Singer) Singer 1973, and *R. hyalinus* (Singer) Thorn et al. 2005. Of these, *R. applicatus, R. dealbatus,* and *R. hyalinus* occur in Brazil (Singer 1943; 1973; 1989; Pegler 1997; Thorn & Barron 1986). We present a description of a new occurrence and a key to the *Resupinatus* species in South America.

Material & methods

Brazilian mangroves extend from 4°30′N to 28°30′S and despite the limited floristic diversity vary significantly in plant growth form, species distribution patterns, and stand structure (Schaeffer-Novelli et al. 1990). From March 2009 to March 2010, 36 field trips were undertaken in four mangroves in Pernambuco state in northeast Brazil: Maria Farinha (07°51′24.8″S34°50′32.7″W), Itamaracá (07°46′52.6″S34°52′53.3″W), Maracaípe (08°32′22.8″S 35°00′29.1″W), and Rio Formoso (08°41′20.8″S 35°06′06.6″W).

After collection, the specimen was analyzed macro- (shape, color, hymenial surface) and micromorphologically (hyphal system, presence/absence and measurements of sterile structures and basidiospores). Microscopical observations were made from slide preparations with 5% KOH, stained with 1% of aqueous phloxine, and Melzer's reagent (Ryvarden 1991). Color designation followed Watling (1969). The material was incorporated to URM.

Scanning electron microscopy (SEM) was conducted at the Laboratório de Microscopia Eletrônica (DF/UFPE). Sections were removed from dried basidiomata and mounted directly on aluminum stubs using carbon adhesive tabs. The fragments were coated with 8–13 nm of gold using a Baltec SCD050 sputter coater and examined with a JEOL JSM-5900 scanning electron microscope.

Taxonomy

Resupinatus poriaeformis (Pers.) Thorn, Moncalvo & Redhead, Mycologia 97(5): 1148 (2006) FIGURE 1–2

≡ *Peziza anomala* var. *poriaeformis* Pers., Syn. meth. fung. 2: 656 (1801)

BASIDIOMATA annual, resupinate, cup-shaped, up to 2–3mm, gregarious, subiculum tomentose to cottony, up to 0.1 mm, grayish (smoke grey 34 to mouse grey 35), without reaction in KOH. HYMENIAL SURFACE smooth, dark grey (drab 33 to mouse grey 35). HYPHAL SYSTEM monomitic; generative hyphae hyaline, clamped, thin-walled. BASIDIA hyaline, clavate, guttulate, 25–30 × 5–7 µm, 4-sterigmata. CYSTIDIA absent. DENDROHYPHIDIA present. BASIDIOSPORES globose, hyaline, thin-walled, smooth, inamyloid, acyanophilous, 4.5–5 µm in diam.

MATERIAL EXAMINED: **BRAZIL. PERNAMBUCO**: Mangrove of Maria Farinha, on dead *Rhizophora mangle* L. (*Rhizophoraceae*), 28.VI.2009, G.S. Nogueira-Melo et al. NM006 (URM 82257).

REMARKS: *Resupinatus poriaeformis* is characterized by small grey cup-shaped basidiomata and globose basidiospores (4.5–5 µm diam., subglobose and ≤ 6 µm in the literature). The species resembles *R. hyalinus* based on the basidioma size and smooth hymenial surface. However, the basidiospores of *R. hyalinus* are elliptical (6–6.5 × 3–3.4 µm). The other species reported from South America have larger basidiomata (0.5–4 cm) and lamellate hymenial surface. Additionally, the basidiospores are ellipsoid (5.8–7.8 × 2.7–3.6 µm) in *R. alboniger*, cylindrical (5–8 × 2.5–3 µm) in *R. dealbatus*, and subglobose (4.4–6

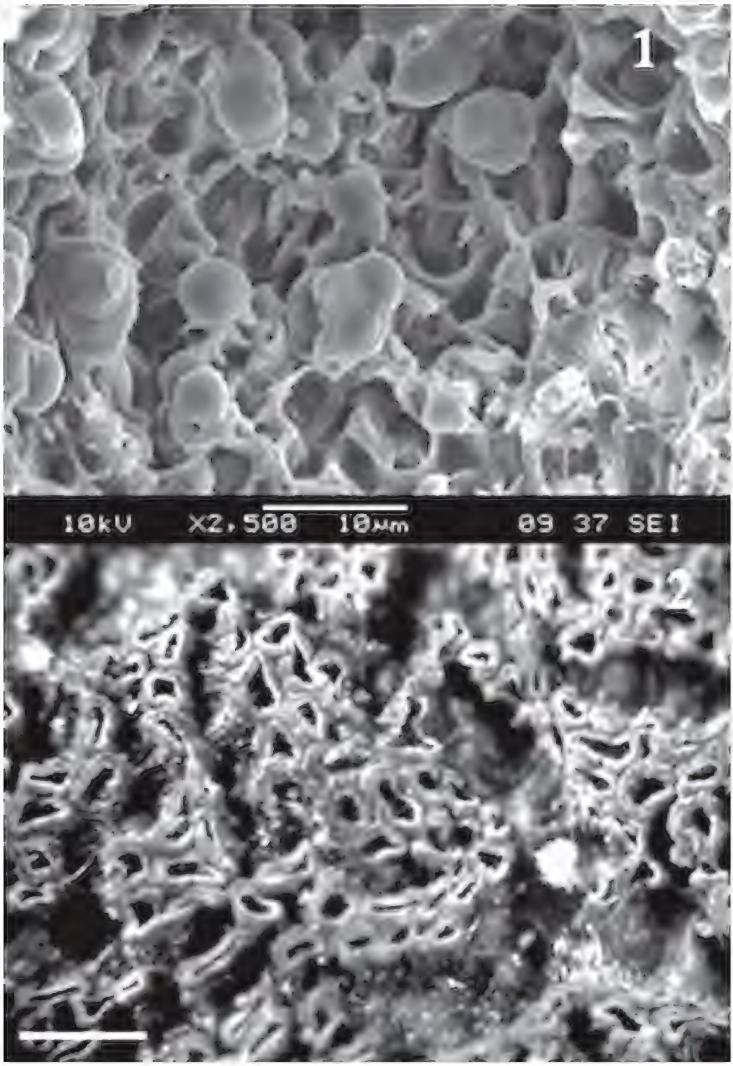

Fig. 1–2. *Resupinatus poriaeformis.* 1. Basidiospores, 2. Basidiomata. Scale bar = 2 mm.

× 4–4.8 µm) in *R. applicatus* (Corner 1981; Ryvarden & Gilbertson 1993; Singer 1943, 1962; Thorn et al. 1986).

Distribution — Ryvarden & Gilbertson (1993) and Bernicchia (2005) report this species as cosmopolitan. Records are known from Honduras, Italy, New Zealand, Puerto Rico, South Africa and Sweden (Cunningham 1963;

Ryvarden 1988; Ryvarden & Gilbertson 1993; Pennycook & Galloway 2004; Thorn et al. 1986, 2006; Bernicchia 2005, O database).

Key to the *Resupinatus* species recorded from South America

1a. Hymenial surface lamellate 2
1b. Hymenial surface smooth 5
2a. Basidiomata reniform to flabelliform up to 2.5–4 cm ..... *R. dealbatus*
2b. Basidiomata cupulate to flabelliform up to 1 cm 3
3a. Cystidial elements lacking ..... *R. graminum*
3b. Cystidial elements present 4
4a. Basidiospores subglobose, 4–4.5 x 5 µm ..... *R. applicatus*
4a. Basidiospores ellipsoid to cylindrical, slightly curved 5.8–7.8 × 2.7–3.6 µm ..... *R. alboniger*
5a. Basidiospores globose to subglobose, 4.5 × 6 µm diam. ..... *R. poriaeformis*
5b. Basidiospores ellipsoid, 6–6.5 × 3.3–4 µm ..... *R. hyalinus*

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New recombinations in *Glomeromycota*

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Abstract — Combined morphological and molecular biological analyses have achieved major advances in the taxonomy of fungal species in phylum *Glomeromycota*. In this study, we analyzed which species might not yet be attributed to their correct genus, focusing on *Acaulospora myriocarpa*, *A. undulata*, *A. nicolsonii,* and *Scutellospora nodosa*. Based on spore wall structure and phylogenetic support, we recombined the taxa as *Archaeospora myriocarpa*, *Ar. undulata*, *Ambispora nicolsonii,* and *Cetraspora nodosa*.

Key words — molecular phylogeny, rDNA

Introduction

Combined morphological and molecular phylogenetic analyses have thoroughly changed the classification of the phylum *Glomeromycota* (e.g. Morton & Redecker 2001, Oehl et al. 2008, 2011a). Today, there are three glomeromycotan classes, five orders, 14 families, and 29 genera (Oehl et al. 2011a,d). When species were attributed to these newly established higher taxa, it was known that some species were incorrectly placed due to insufficient available morphological or phylogenetical information (Oehl et al. 2008, 2011a,b,e). In this paper, we consider four of those species —*Acaulospora myriocarpa*, *Ac. undulata*, *Ac. nicolsonii,* and *Scutellospora nodosa*. As a result of new available information, we transfer these species to their proper genera based on their morphological features.

Material & methods

Specimens

Holotype or isotype materials of *Ac. myriocarpa* (OSC, herbarium of Oregon State University, Corvallis, U.S.A.), *Ac. undulata* (OSC), and *Sc. nodosa* (DPP, the Plant Pathology herbarium, Academy of Agriculture, Szczecin, Poland) were analyzed. Type material of *Ac. nicolsonii* (Walker et al. 1984) was either never deposited at OSC or lost during shipping (Richard Halse & Joey Spatafora, curators of OSC, pers. communication); therefore here we rely on the reinterpretation by Walker et al. (1984) of the original species description and illustrations and on comparison of *Ac. nicolsonii* with the type materials of *Ambispora gerdemannii* and *Am. fennica* in Walker et al. (2007).

Morphological analyses

Morphological descriptions of spores (including sporiferous saccules, sporogenous cells, germination shields, and subcellular structures) are based on observations of specimens mounted in polyvinyl alcohol-lactic acid-glycerol (PVLG) and in a mixture of PVLG+Melzer's reagent (Brundrett et al. 1994). Spore structure terminology follows Goto & Maia (2006), Sieverding & Oehl (2006), Oehl et al. (2006), Spain et al. (2006), Palenzuela et al. (2008, 2010, 2011), Goto et al. (2008) and Oehl et al. (2011a–c) for acaulosporoid, ambisporoid, archaeosporoid, entrophosporoid, and gigasporoid spore forming species.

Results

Acaulospora myriocarpa and *Acaulospora undulata*

Both *Ac. myriocarpa* and *Ac. undulata* form bi-walled spores laterally on the neck of a sporiferous saccule. The outer wall is bi-layered and the inner wall triple-layered. The staining reaction of the mycorrhizal structures is faint to absent, and vesicle formation has been observed only infrequently in pure cultures of the two species (Schenck et al. 1986) and then possibly due to misinterpretation (Sieverding own observations). These features attribute both species unequivocally to the genus *Archaeospora* J.B. Morton & D. Redecker emend. Spain (Spain 2003).

Archaeospora myriocarpa (Spain, Sieverd. & N.C. Schenck) Oehl, G.A. Silva, B.T. Goto & Sieverd., **comb. nov.**

MycoBank MB 561657

≡ *Acaulospora myriocarpa* Spain, Sieverd. & N.C. Schenck, Mycotaxon 25: 112. 1986.

Archaeospora undulata (Sieverd.) Sieverd., G.A. Silva, B.T. Goto & Oehl, **comb. nov.**

MycoBank MB 561662

≡ *Acaulospora undulata* Sieverd. Angew. Bot. 62: 373. 1988.

Acaulospora nicolsonii

Acaulospora nicolsonii regularly forms spores on the lateral branch (i.e. pedicel) of a sporiferous saccule. Its spores have three walls: an outer wall, which during degradation shows cracking fissures known only for *Ambispora*

species, a middle wall, and an inner wall. Since the spore morphology exactly matches the features of typical acaulosporoid spores in *Ambispora* C. Walker et al. (= 'acaulo-ambisporoid' spores), we transfer the species to this genus (Spain et al. 2006, Walker et al. 2007, 2008).

Ambispora nicolsonii (C. Walker, L.E. Reed & F.E. Sanders) Oehl, G.A. Silva, B.T. Goto & Sieverd., **comb. nov.**

MycoBank MB 561663

≡ *Acaulospora nicolsonii* C. Walker, L.E. Reed & F.E. Sanders, Trans Br. Mycol. Soc. 83: 360. 1984.

Scutellospora nodosa

New observations on the germination shield of the type material of *Sc. nodosa* reveal that the shields are hyaline and multiply lobed, which is typical for *Racocetraceae* species (e.g. Oehl et al. 2010). Since the species has triple-walled spores (Blaszkowski 1991, Oehl et al. 2008), we transfer it to the genus *Cetraspora* Oehl et al. (*Racocetraceae*).

Cetraspora nodosa (Błaszk.) Oehl, G.A. Silva, B.T. Goto & Sieverd., **comb. nov.**

MycoBank MB 561664

≡ *Scutellospora nodosa* Błaszk., Mycologia 83: 537. 1991.

Discussion

Archaeospora has now three species— two with smooth spore walls (*Ar. trappei, Ar. myriocarpa*) and one with an ornamented (undulating) spore surface (*Ar. undulata*).

Ambispora has now three species (*Am. callosa*, *Am. fecundispora, Am. leptoticha*) for which only glomoid spore formation is known and two species (*Am. jimgerdemannii, Am. nicolsonii*) for which only acaulosporoid spore formation is known. All other *Ambispora* species have spore bimorphism (*Am. appendicula*, *Am. brasiliensis*, *Am. fennica*, *Am. gerdemannii, Am. granatensis*) (Spain et al. 2006, Walker 2008, Goto et al. 2008, Palenzuela et al. 2011).

Three major characteristics strongly support transfer of *Ac. nicolsonii* to *Ambispora*: i) the presence of three walls (outer, middle, inner), ii) the clear pedicel on the sporiferous saccule neck, referred to as a 'stalk' in the protologue (Walker et al. 1984), and iii) outer spore walls that have the typical 'cracking fissures' as known for outer walls of almost all known *Ambispora* species, e.g. *Am. appendicula*, *Am. gerdemannii*, *Am. fennica,* and *Am. brasiliensis* (see illustrations in Walker et al. 1984; Spain et al. 2006, Goto et al. 2008). The absence of fracturing (i.e., 'plate-like splitting') of the middle wall layers in *Am. nicolsonii* (Walker et al. 2007) might be the most important morphological difference between it and *Am. gerdemannii* and *Am. fennica*, while the outer walls in both *Am. nicolsonii* and *Am. gerdemannii* do not stain in Melzer's reagent but do in *Am. fennica* (Walker et al. 2007; Oehl, pers. observation).

The recent transfer of *Am. brasiliensis* to *Acaulospora* by Krüger et al. (2011) cannot be accepted; the spore morphology of the Scottish material described and illustrated as *Acaulospora brasiliensis* by Krüger et al. (2011) does not match the morphology of *Am. brasiliensis*, and their sequenced material apparently represents a true *Acaulospora* species rather than authentic *Am. brasiliensis*. Krüger et al. (2011) unfortunately ignored the major characters elaborated by Spain et al. (2006) for *Ambispora* species and detailed by Goto et al. (2008) for *Am. brasiliensis*.

There have been doubts about the shield morphology of *Cetraspora nodosa* (*Scutellospora nodosa* in Oehl et al. 2008), and sequences of the fungus deposited in public databases during more than 10 years were apparently misidentified. New combined morphological and molecular phylogenetic analyses, based on identified sequences recently placed in the public databases, now show a congruency between morphological and molecular data supporting *C. nodosa* as closely related to *C. helvetica*, *C. pellucida,* and *C. gilmorei* (Oehl et al. 2010, 2011b) within the monophyletic genus *Cetraspora*.

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Pouzarella (*Agaricales, Entolomataceae*) species from New South Wales (Barrington Tops National Park) and northeastern Queensland, Australia

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Abstract —Ten *Pouzarella* species are described from New South Wales and northern Queensland, Australia, of which eight (*P. albostrigosa*, *P. farinosa*, *P. fusca*, *P. lageniformis*, *P. pamiae*, *P. parvula*, *P. pilocystidiata* and *P. setiformis*) are new to science and two (*P. debilis* and *P. lasia*) are reported for the first time for Australia. Phylogenetic analyses of the mitochondrial small subunit rRNA (mtSSU), two variable domains (D1, D2) of the 25-28S large subunit rDNA (LSU), and a portion of the second largest subunit of the RNA polymerase (RPB2) gene support separation of these species into three clades. Morphological characters common to these clades are described, and a key to species based on the characters that are distinct between clades is included.

Key words — *Basidiomycota*, phylogeny, mtSSU, LSU, RPB2

Introduction

The genus *Pouzarella* Mazzer (*Entolomataceae, Agaricales*) was first monographed on a worldwide basis by Mazzer (1976). He proposed replacing *Pouzaromyces* Pilát with *Pouzarella* because the type species of *Pouzaromyces*, *Agaricus fumosellus* G. Winter, had no extant type material and the protologue description had been interpreted as probably referring to a coprinaceous species (Mazzer 1976, Noordeloos 1984, 1992; see Baroni et al. 2008 regarding nomenclature). The name *Pouzarella* has since been accepted by many researchers (Largent 1994, Karstedt et al. 2007, Baroni & Ortiz 2002, Baroni et al. 2008, Horak 2008).

Pouzarella combines the following features: typically mycenoid basidiomata (often resembling a smallish *Inocybe* (Fr.) Fr.); strongly fibrillose, squamulose, or hispid squamulose pilei; often radially strigose stipe bases; tramal hyphae with externally incrusting pigments; pileipellis and stipitipellis hyphae with parietal and/or externally incrusting pigments; a pileipellis (at least on the pileus center) with erect to semi-erect hyphae composed of chains of cells; nodulose-angular, heterodiametric basidiospores with 6–9 facets; and a hymenium frequently, but not always, with dark brown contents or aborted basidia.

Because there have been no *Pouzarella* cited in Australian publications on *Entolomataceae* Kotl. & Pouzar (Gates & Noordeloos 2007, Gates et al. 2009, Noordeloos & Gates 2009, May & Wood 1997, Grgurinovic 1997), we provide the first reports and descriptions of *Pouzarella* in Australia. Of the ten *Pouzarella* species covered, four are newly described from the Barrington Tops National Park in central New South Wales, while four new species and two new records are cited for the wet tropical rainforests of northeastern Queensland. We also present a phylogeny based on a supermatrix of 1985 characters from three partial gene regions: mitochondrial small subunit (mtSSU), 25–28S nuclear rDNA large subunit (LSU), and a portion of the gene encoding the second largest subunit of RNA polymerase gene (RPB2). The sequence analyses strongly support separation of the ten Australian taxa into three clades, each of which can be diagnosed by micromorphological characters.

Materials & methods

Macromorphological and micromorphological features

Specimens were collected during February–April in 2009 and 2010 from different sites within the Wet Tropics Bioregion in northeastern Queensland and in April 2010 in the Barrington Tops National Park of central New South Wales. Basidiomata collected in the field were stored in plastic containers for transport to the laboratory. Macroscopic features were described from recently collected fresh materials. Field GPS coordinates for each collection were taken using a Garmin GPSmap 60CSx. Colors were described subjectively and coded according to Kornerup & Wanscher (1978), with color plate designations noted in parentheses. Color plate abbreviations itemize page number, column, and row (e.g., 8D-F5-6 = p. 8, columns D–F, rows 5–6.)

Dried specimens were examined microscopically with a trinocular research-grade Nikon Labophot compound microscope fitted with bright field optics according to Largent (1994: 1–3); basidiospore measurements follow Baroni & Lodge (1998: 681). Digital microphotographs were made using a Nikon Coolpix 990 camera focused through the trinocular head of the compound microscope.

All microscopic measurements were obtained using a GTCO Corporation Graphic Digitizer, Model DP5A-111A, connected to a laptop computer. The software utilized, Measure Me 101 v 1.0, was modified for use on a contemporary computer from the BASIC program, Metrics5 (David Malloch, University of Toronto). The mathematical factors determined using this program include: arithmetic mean (x_m) of basidiospore

length and width ± standard deviation for n objects measured; quotient of basidiospore length by width (E) indicated as a range for n objects measured; mean of E values (Q) ± standard deviations. The sample size (n) equals the quotient (n = x/y) of the total number of microscopic structures measured (x) and the number of basidiomata studied (y).

All collections for New South Wales cited in 'Additional collections examined' are deposited in The Plant Pathology Herbarium, Orange Agricultural Institute (DAR); the Queensland collections were split with duplicates deposited in the Australian Tropical Herbarium (CNS) and The Queensland Herbarium (BRI). All holotype and isotype collections are deposited in the herbaria designated in the Latin descriptions using acronyms from Holmgren et al. (1990).

DNA sequences and phylogenetic analyses

Lyophilized basidiomata were pulverized (4 mps for 20 sec) with glass beads (FastPrep FP120 homogenizer, QBiogene, Carlsbad CA USA). DNA was extracted with 2 × cetyl trimethylammonium bromide (CTAB) buffer, purified with phenol-chloroform-isoamyl alcohol (25:24:1) bound to silica columns (GeneClean Turbo Columns, MP Biomedicals, Solon OH USA), and washed with 70% ethanol (Baumgartner et al. 2010). Portions of three loci were amplified using Polymerase Chain Reaction (PCR). PCR primers used were MS1 and MS2 for mtSSU (White et al. 1990); Ctb6 and Tw13 for nuclear rDNA LSU (variable domains D1 and D2; White et al. 1990); and for the RPB2 portion either pb2i6f and rpb2i7r (Co-David et al. 2009) or our *Pouzarella*-specific primers, rpb2-PouF (5' to 3' - GAA GGT CAA GCT TGT GGT C) and rpb2-PouR (5' to 3' - CAT GCT YGG ATG GAT TTC).

The mtSSU and LSU genes were amplified in 25 µL reactions containing 1× GoTaq Flexi Buffer (Promega, Madison WI USA), 2 mM $MgCl_2$, 0.2 mM dNTPs, 500 nM forward and reverse primers, 0.2 mg/mL bovine serum albumin, 0.2 M betaine, 0.025 U Taq polymerase, and 1–2 µL of diluted template DNA. RPB2 was optimally amplified according to the primer pair used. The rpb2-PouF and rpb2-PouR primer-based amplifications utilized 25 µL reactions using the above protocol, except that betaine was increased to 1 M. The Co-David et al. (2009) based RPB2 amplifications increased the concentrations of primers rpb2-i6f and rpb2-i7r to 1 µM and the betaine concentration to 1 M. PCR cycling conditions were as follows: mtSSU and LSU: 94°C for 1 min, followed by 40 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, followed by a final extension of 72°C for 7 min. RPB2: EITHER the touchdown protocol using primers rpb2-i6f and rpb2-i7r (Co-David et al. 2009) OR [*Pouzarella* primers rpb2-PouF, rpb2-PouR] 95°C for 5 min, followed by 40 cycles of 95°C for 30 sec, 50°C or 52°C for 1 min 30 sec, and 72°C for 1 min, followed by a final extension of 72°C for 7 min. Unresolved sequences with overlapping chromatograms were resolved by sub-cloning PCR products with the TOPO TA cloning kit (Invitrogen, Carlsbad CA USA) according to Bergemann et al. (2009).

PCR products were cleaned using 1 µL of ExoSAP-IT (GE Healthcare, Pittsburgh, PA USA). Sequencing reactions were carried out in 10 µL reactions containing 1 µL of Applied Biosystems BigDye ver. 3.1 (Applied Biosystems, Carlsbad CA USA), 0.4 µm primer, 0.875 × Sequencing Buffer (Applied Biosystems, Carlsbad CA USA) and 1 µL PCR water. Cycling parameters followed: 39 cycles of 96°C for 10 sec, 50°C for 5 sec,

60°C for 4 min with an infinite hold at 15°C. Reactions were purified with 3 M NaOAc, 250 mM EDTA (pH 8) and ethanol by centrifugation at 2500 G. Reactions were washed once with 70% EtOH and centrifuged at 2500 G for 15 min. Precipitated products were stored at –20°C.

Forward and reverse sequences for each locus were generated on an Applied Biosystems 3130xl Genetic Analyzer at Middle Tennessee State University. Sequence contigs were edited in Sequencher 4.8 (GeneCodes Corp., Ann Arbor, MI USA) and manually aligned in SE-Al (Rambaut 2002). Prior to tree estimation, the model selection feature in TOPALi v. 2.5 (Milne et al. 2004) was used to test for the best-fit model of nucleotide substitution using three selection criteria (AIC1 = Akaike information criterion, AIC2 = second order correction and BIC = Bayesian information criterion). Each criterion selected general time-reversible (GTR) as the best fitting substitution model. All phylogenetic analyses were conducted on the combined dataset (mtSSU + LSU + RPB2) using *Lyophyllum decastes* (Fr.) Singer, *L. leucophaeatum* (P. Karst.) P. Karst., and *Calocybe carnea* (Bull.) Donk as outgroups and including two additional taxa (*Entoloma violaceovillosum* Manim. & Noordel. and *E. araneosum* (Quél.) M.M. Moser) known to have typical *Pouzarella* features (Co-David et al. 2009). Maximum likelihood (ML) analyses implemented in TOPALi v. 2.5 (Milne et al. 2004) used a heuristic search algorithm based on a rapid branch swapping operation (PhyML; Guindon & Gascuel 2003). The level of clade support was assessed using 100 bootstrap (BS) replicates. Bayesian inferences (BI) were implemented in MrBayes ver. 3.1.2 (Ronquist & Huelsenbeck 2003) using the GTR set to two runs of one million generations sampling every 100 generations. After convergence, Bayesian posterior probabilities (BPP) were determined after calculating a 50% majority rule consensus tree.

GenBank accession numbers for each sequence are listed at the end of the type description for each species along with the habitat data and GPS co-ordinates of the holotype collection. Sequences for all other collections are listed after the collection number in the 'Other collections examined' section. GenBank accession numbers for other species included in the phylogenetic analyses are as follows (mtSSU, LSU, and RPB2, respectively): *Entoloma violaceovillosum* (GQ289345, GQ289205, GQ289273), *E. araneosum* (GQ289293, GQ289153, GQ289225), *Calocybe carnea* (AF357097, AF223178, DQ367432), *Lyophyllum leucophaeatum* (AF357101, AF223202, DQ367434), and *L. decastes* (AF357136, AF042583, DQ367433).

Results

We obtained sequences for all three loci (mtSSU, LSU, RPB2) for 22 *Pouzarella* collections except that we omitted one RPB2 sequence (for *P. parvula* DL Largent 9901) after several amplification attempts failed to produce PCR amplicons that could be sequenced. The dataset comprised 686 (mtSSU), 661 (LSU), and 638 (RPB2) aligned characters assembled into a supermatrix consisting of 1985 characters. Of these, 122 mtSSU, 61 LSU, and 104 RPB2 characters were excluded at the 5' or 3' ends due to heterogeneity in the lengths of the sequences obtained for different samples.

We conducted ML and BI analyses on the combined dataset. As the two tree topologies were fully congruent, we present only the ML tree with corresponding

BPP values included for comparison. Most branches are strongly supported by BS values greater than 70% and BPP values greater than 0.95, although in some cases, high levels of BPP support (0.99–1.00) were found when BS values were less than 70%. The Australian collections formed three clades (Plate 21): Clade I formed a well-supported (BS >70, BPP = 1.00) group comprising five species (*Pouzarella lasia*, *P. albostrigosa, P. fusca, P. debilis, P. setiformis*). Clade II included two species (*P. pamiae, P. farinosa*) with high BPP (0.99) but low BS (<70%) support. Clade III formed a well-supported group (BS >70, BPP = 1.00) of three species (*P. pilocystidiata, P. parvula, P. lageniformis*). The clades can be distinguished using micromorphological features (cheilocystidia, aborted basidia, and pileocystidia; see Key). The Australian taxa are not resolved as monophyletic in relation to the two European taxa included (*E. araneosum* and *E. violaceovillosum*), though the grouping of the Australian clade III with these species received low BS (but high BPP) support.

Taxonomy

1. ***Pouzarella pilocystidiata*** Largent & Skye Moore, **sp. nov.** Plates 1–2

MycoBank MB 519567

Habitu Pouzarellae fulvostrigosae *similis, sed centro pilei hispido-squamuloso, atrofusco, cheilocystidiis versiformibus, incrustationibus externis in hyphis tramalibus distinctis valdis, caulocystidiis copiosis, pseudocystidiis nullis atque superficie pilei argentea differt.*

Type — Australia, New South Wales, central Hunter District, Barrington Tops National Park, end of Blue Gum Loop Track, 32°08′31.7″S 151°30′56.4″E, 366.4 m, 22 April 2010, DL Largent 9932 (**holotype** DAR), sequences: HQ876543 (mtSSU), HQ876521 (LSU), HQ876500 (RBP2).

Etymology — Derived from the Latin *pilus* (= hair) + *cystidium*, referring to the long pileocystidia.

Pileus 8–19 mm broad, 2–7 mm high, convex or conic-campanulate and suggestively umbonate becoming broadly convex and non-umbonate with expansion, densely hispid-squamulose on the disc, hispid-squamulose to squamulose near the disc, matted appressed squamulose to appressed fibrillose elsewhere, with age the marginal area becomes orange-white to orange-gray (5-6A-B2) as the trama is exposed through separation of the cuticle; squamules and fibrils dark brown (5-7E-F5-6) on dark blond to brown (5E-F4) background, dull, opaque and not hygrophanous; margin decurved at all times, not translucent, even. Trama less than 1 mm thick, dark brown. Taste farinaceous. Odor mild or somewhat pungent. Lamellae 3–8 mm long, 1–5 mm high, narrow when young, moderately broad, broad or ventricose when mature, at first light brownish-orange (6C4 camel) then orange-white to orange-gray or reddish-white to orangish-gray (5-7A-B2-3), with age developing brown, irregularly arranged spots, adnate to short decurrent or with a distinct decurrent tooth,

close then subdistant; margin whitish and ± fimbriate because of cystidia, becoming eroded with age, bruising brownish with handling. Lamellulae 3 (2 short, 1 medium to medium-long) between lamellae. Stipe 23–45 mm long, 0.5–2 mm broad, equal, dark brown (5-7E-F4-6), darkens upon handling to dark brownish-gray to grayish-brown (7F2-3), at first squamulose-hispid with erect squamulose tips at the apex, elsewhere entirely covered with entangled squamules and fibrils, with age, rain or handling the squamules and fibrils get plastered on the surface and thus, the stipe appears fibrillose; stipe base with orange cinnamon strigose hairs. Bruising reaction absent.

Basidiospores nodulose angular with 6–8 angles, 9.2–14.6(–15.1) × 5.4–8.7 (–9.7) µm (x_m = 11.6 ± 1.1 × 7.2 ± 0.7 µm; E = 1.22–2.08; Q = 1.63 ± 0.16; n = 118/4). Basidia 4-sterigmate, cylindro-clavate to clavate, moderately long to long, 32.5–50.1 × 6.3–12.7 µm (x_m = 40.8 ± 4.4 × 10.2 ± 1.6 µm; E = 2.90–6.27; Q = 4.12 ± 0.81; n = 34/4). Cheilocystidia abundant, colorless, thin-walled, versiform (clavate, acuminate, aciculate, ventricose-rostrate), most common near the pileal margin and on the lamellulae, 39.5–82.8 × 4.3–24.0 µm (x_m = 57.8 ± 11.4 × 13.5 ± 4.3 µm; E = 2.27–12.34; Q = 4.90 ± 2.45; n = 24/4). Pleurocystidia absent. Pseudocystidia absent. Aborted Basidia absent. Hyphae of lamellar trama very long, similar in size to *Inocephalus* (Noordel.) P.D. Orton, 164.1–748.0 × 3.8–26.8 µm. Pileipellis 45–400 µm deep, composed of entangled strands of thick-walled hyphae in chains of up to 10 cells, entirely erect and trichodermial when young, upon pileal expansion remaining so in the center, semi-erect near the disc and repent at the margin. pileocystidia cylindric, cylindro-clavate, narrowly obclavate, 28.1–171.3 × 6.8–20.1 µm (x_m = 79.7 ± 25.7 × 11.3 ± 3.6 µm; E = 3.56–10.81; Q = 7.16 ± 2.40; n = 19/3). Hyphae of the pileal trama similar in length and width to the lamellar trama. Stipitipellis loosely entangled layer of hyphae, 45–205 µm deep. Caulocystidia cylindric to cylindro-clavate 53.2–125.4 × 4.5–9.4 µm. Oleiferous hyphae absent. Lipoid bodies absent. Pigmentation with strong, external incrustations in the pileal, lamellar and stipe tramas; parietal as internal incrustations in the pileipellis and stipitipellis.

Ecology and distribution: Solitary or scattered in soil either amongst leaf litter or under a log in amongst rocks, subtropical and temperate gallery rainforests; central Hunter district, New South Wales.

Additional collections examined —**AUSTRALIA. New South Wales**, central Hunter District, Barrington Tops National Park, Williams Day Use Area, end of blue gum track, 32°09′03.0″S 151°31′28.2″E, 366.4 m, 8 April 2010, DL Largent 9848 (mtSSU HQ876542, LSU HQ876520, RPB2 HQ876499); Pool of Reflections track, 32°08′29.5″S 151°30′55.0″E, 366.4 m, 20 April 2010, DL Largent 9921; Lion's Rock Track, 32°09′10.2″S 151°3′38.6″E, 366.4 m, 26 April 2010, DL Largent 9949 (mtSSU HQ876544, LSU HQ876522, RPB2 HQ876501).

Comments— *Pouzarella pilocystidiata* is diagnosed by a pileus with a squamulose-hispid disc and elsewhere appressed squamulose to appressed

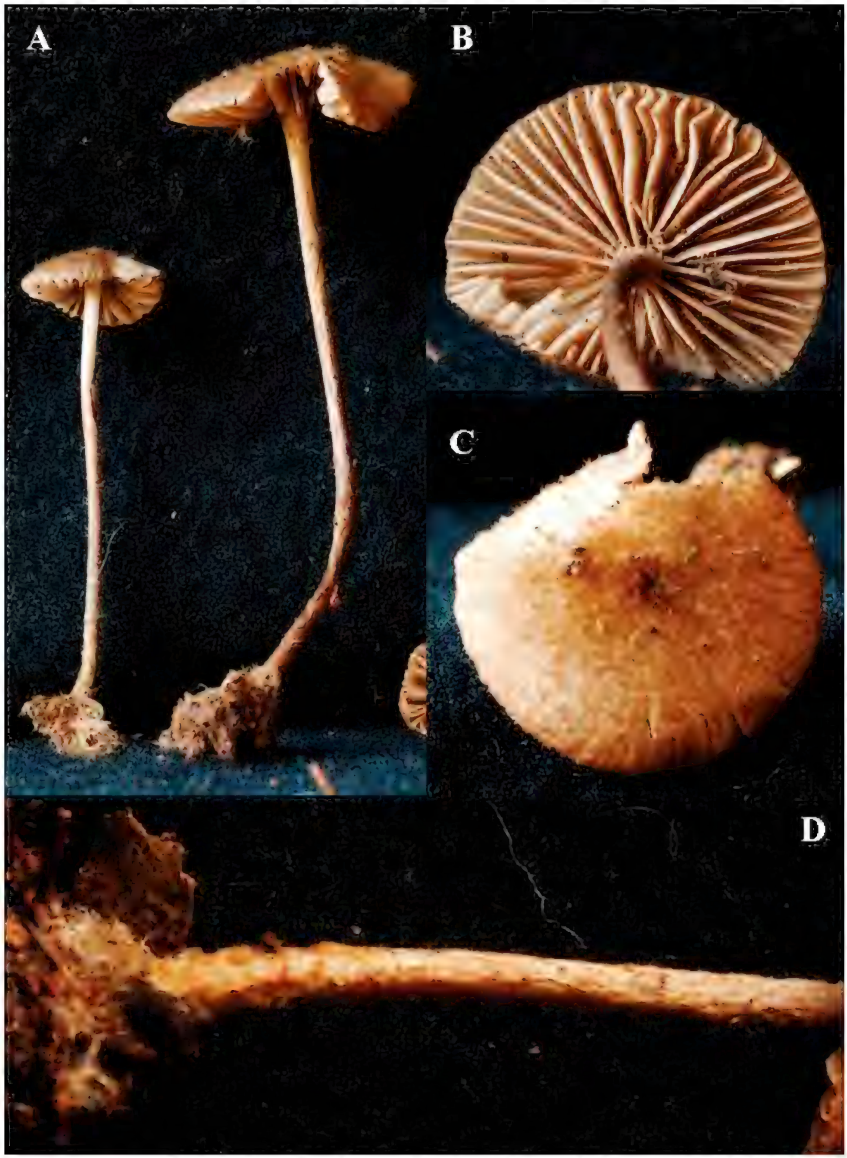

PLATE 1 – *Pouzarella pilocystidiata*. A: Basidiomata (DLL 9932, HOLOTYPE); B: Lamellae (DLL 9932); C: Pileus surface (DLL 9932); D: Strigose stipe base, stipe surface (DLL 9949).

fibrillose, a tomentulose to densely hirsute stipe apex, a dark brown stipe that bruises dark grayish-brown with a strigose base with light-colored to orange cinnamon hairs, a farinaceous taste, basidiospores generally smaller than 12.0

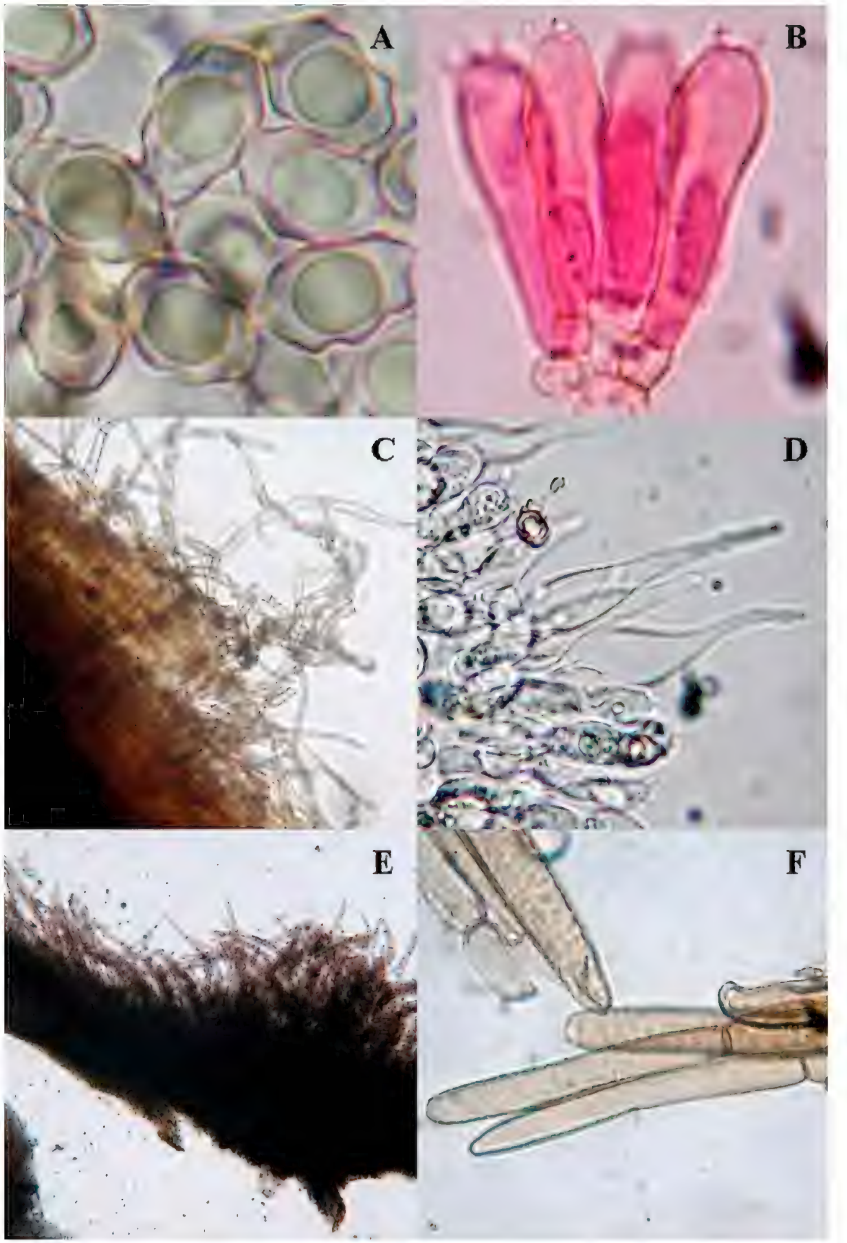

Plate 2 – *Pouzarella pilocystidiata*. A: Basidiospores (1000×) (DLL 9949); B: Basidia (400×) (DLL 9959); C: Stipitipellis (100×) (DLL 9949); D: Cheilocystidia (400×) (DLL 9932, holotype); E: Pileipellis on and near disc (100×) (DLL 9921); F: Pileocystidia (400×) (DLL 9949).

× 7.5 µm, basidia narrower than 11.0 µm, pileipellis and stipitipellis ≤400 µm thick, and ≤175 µm long cylindro-clavate pileocystidia and caulocystidia.

Pouzarella pilocystidiata morphologically resembles *P. fulvostrigosa* (Berk. & Broome) Mazzer, which is distinguished by a densely silvery fibrillose mouse gray pileus, lageniform cheilocystidia, minutely incrusted tramal hyphae, scattered pseudocystidia, and caulocystidia (Noordeloos 1979).

Australian taxa similar to *Pouzarella pilocystidiata*

Pouzarella pilocystidiata shares with *P. lageniformis* and *P. parvula* nodulose angular basidiospores (x_m <13.5 µm long), externally incrusted tramal hyphae, abundant, thin-walled, versiform (rostrate, ventricose, lageniform) cheilocystidia, long cylindro-clavate pileocystidia, and the absence of aborted basidia. Morphological features unique to *Pouzarella pilocystidiata* include the small hispid-squamulose dark brown/dark blond pileus, densely hirsute stipe surface, farinaceous taste, basidiospore size, rare acuminate pileocystidia, abundant cylindro-clavate caulocystidia, and lack of setiform pileocystidia. *Pouzarella lageniformis* is differentiated by a 11 × 2 mm hispid-squamulose orange-white/grayish orange pileus, entirely pruinose stipe surface, mild taste, slightly larger basidiospores, acuminate pileocystidia, and lack of setiform and aculeate thick-walled caulocystidia. *Pouzarella parvula* differs by its smaller matted squamulose pileus, adnexed lamellae, glabrous stipe, larger basidiospores, versiform (some rostrate ventricose) cheilocystidia, entangled pileipellis, and lack of aborted basidia and caulocystidia.

2. ***Pouzarella lageniformis*** Largent & Skye Moore, **sp. nov.** PLATES 3–4

MYCOBANK MB 519568

A speciebus congenericis omnibus ceteris pileo juventute omnino hispido-squamuloso, superficie pilei omnino pallide griseo-brunnea squamulis aurantiaco-albis ornata, apice stipitis pruinoso, alibi stipite fasciculis fibrillarum rarenter praedito, basidiosporis 10.4–13.8 × 6.6–8.7 um, mediane 12.06 × 7.55 um, basidiis mediane 36.9 × 11.9 um, pileocystidiis clavatis acuminatis vel aculeatis, cheilocystidiis versiformibus aliquibus rostrato-ventricosis, caulocystidiis aculeatis differt.

TYPE — Australia, New South Wales, central Hunter District, Barrington Tops National Park, Williams Day Use Area, end of Blue Gum Track, 32°09′01.9″S 151°31′28.7″E, 365.5 m, 18 April 2010, DL Largent 9895 (**holotype** DAR), sequences: HQ876545 (mtSSU), HQ876523 (LSU), HQ876502 (RBP2).

ETYMOLOGY — Derived from the Latin *lagena* (= flask) + *forma* (= shaped), referring to the flask-shaped or rostrate ventricose cheilocystidia.

PILEUS 11 mm broad, 2 mm high, broadly convex, when young entirely erect hispid-squamulose with the squamules somewhat scattered, when mature remaining so on and near the center, semi-erect near the center, repent elsewhere, squamules orange-white (5-6A1-2), background light grayish-brown (6C-D3), overall grayish-orange (6B-C2), dull, dry, opaque, not hygrophanous,

Plate 3 – *Pouzarella lageniformis* (DLL 9895, holotype)
A: Basidiomata; B: Lamellae; C: Pileus surface; D: Stipe surface.

striate with striae colored as the background. Margin decurved, fringed in places. Taste and Odor indistinct. Lamellae 5 mm long, 1.5 mm high, broad and ventricose, short-decurrent, orange-gray (5A2), light-colored when dried, subdistant, margin suggestively and minutely serrulate, whitish. Lamellulae 3 (2 short, 1 medium to medium long) between lamellae. Stipe 33 mm long, 1 mm broad at apex, 0.75 mm broad at base, slightly tapered, entirely pruinose with the pruinae scattered and orange-white to orange-gray on a grayish-brown (6D3) background, dull, hollow; stipe base densely pruinose. Bruising reaction absent.

Basidiospores angular-nodulose with 6–8 angles, heterodiametric in profile view, 6-angled and isodiametric in polar view, 10.4–13.8 × 6.6–8.7 µm (x_m = 12.1 ± 1.0 × 7.6 ± 0.6 µm; E = 1.38–1.87; Q = 1.6 ± 0.14 (heterodiametric); n = 29/1). Basidia 4-sterigmate, sterigmata 0.7–3.5 µm long, base of basidia 3.3–5.8 µm wide, relatively small, full of small droplets, 29.3–43.6 × 9.9–14.2 µm (x_m = 36.9 ± 3.3 × 11.9 ± 1.4 µm; E = 2.56–4.32; Q = 3.13 ± 0.48; n = 13/1). Cheilocystidia abundant, scattered, versiform (long ventricose-rostrate, acuminate), 47.5–133.6 × 8.7–28.8 µm (x_m = 85.6 ± 22.4 × 17.9 ± 6.0 µm;; E = 3.16–10.75; Q = 5.24 ± 2.28; n = 10/1). Pleurocystidia absent. Pseudocystidia absent. aborted basidia absent. Hyphae of lamellar trama relatively long 230.2–418.7 × 11.3–31.4 µm. Pileipellis 117–333 µm deep, composed of loosely entangled, long strands of hyphae with chains of 3–5 cells, individual cells relatively long, entirely erect to semi-erect and trichodermial in the center when young, upon pileal expansion remaining so in the center, becoming semi-erect near the center and repent elsewhere. Pileocystidia very long, clavate, aculeate, or acuminate, 105.4–192.7 × 10.7–20.2 µm. Hyphae of the pileal trama 194.6–451.3 × 9.4–17.1 µm. Stipitipellis 30–150 µm deep, composed of scattered strands of thick-walled hyphae. Caulocystidia aculeate, thick-walled, 20.5–144.4 × 5.0–9.2 µm. Pigmentation externally incrusted on the tramal hyphae of the pileus, stipe, lamellae, parietal with internal incrustations in the pileipellis and stipitipellis.

Ecology and distribution: Solitary amongst leaf litter, subtropical gallery rainforest; known only from type locality.

Comments— *Pouzarella lageniformis* is diagnosed by an entirely hispid squamulose young pileus with orange-white squamules on a light grayish brown background, stipe with a pruinose apex and rare fibrillose bundles elsewhere, basidiospores averaging 12.1 × 7.6 µm, basidia generally >11.0 µm long, clavate, acuminate, or aculeate pileocystidia, versiform (some rostrate ventricose) cheilocystidia, and aculeate caulocystidia.

See *P. pilocystidiata* for comparisons with Australian and other taxa related to *P. lageniformis*.

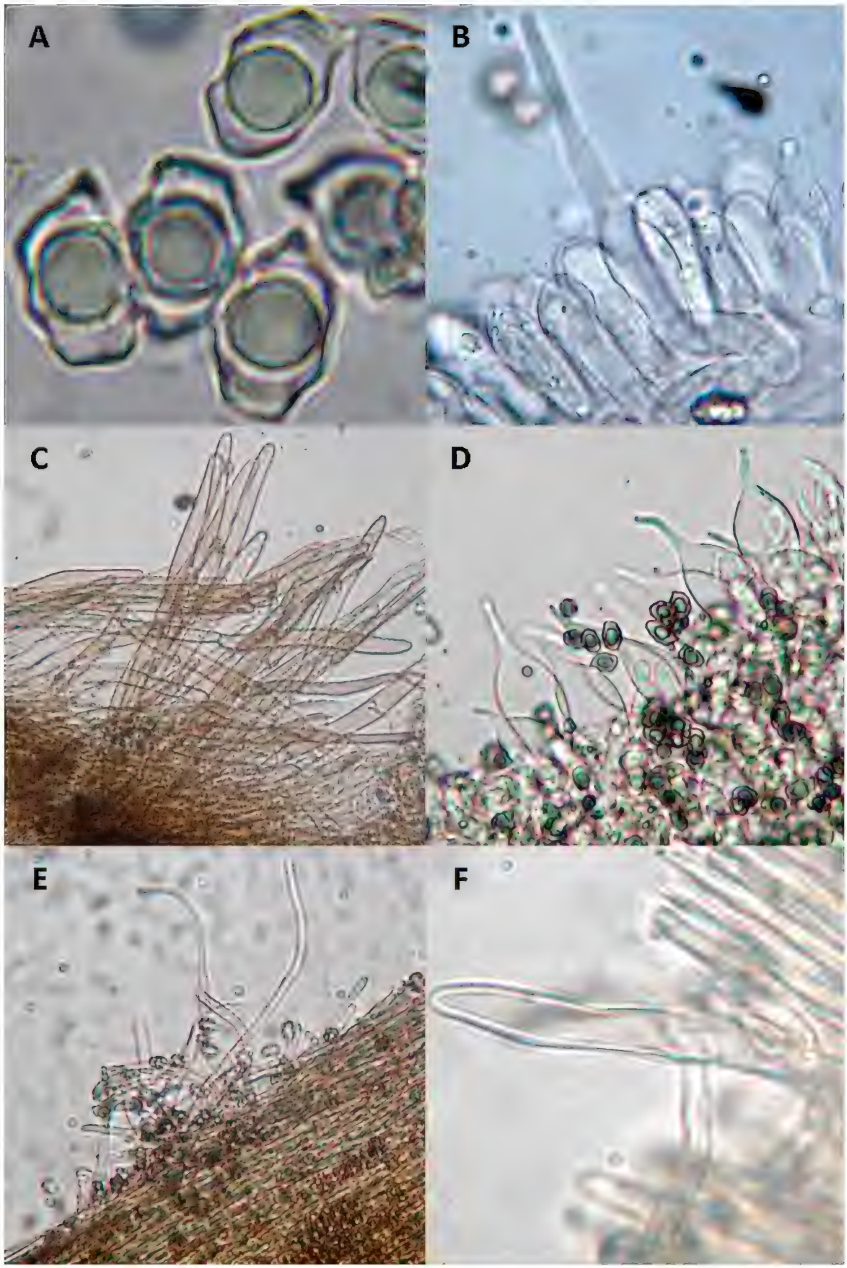

Plate 4 – *Pouzarella lageniformis* (DLL 9895, holotype). A: Basidiospores (1000×); B: Basidia and narrow rostrate-ventricose cheilocystidia (400×); C: Pileocystidia and pileipellis (180×); D: Cheilocystidia (100×); E: Stipitipellis (100×); F: Thick-walled caulocystidium (400×).

3. ***Pouzarella parvula*** Largent & Skye Moore, **sp. nov.** PLATES 5–6

MYCOBANK MB 519569

A speciebus congenericis omnibus ceteris pileo parvo, 6 mm lato, 2 mm alto, implexo, squamuloso, lamellis adnexis, stipite glabro, basidiosporis mediane 13.23 × 7.7 um, cheilocystidiis versiformibus aliquibus rostrato-ventricosis, pileipelle implexa, pseudocystidiis caulocystidiisque nullis differt.

TYPE — Australia, New South Wales, Central Hunter District, Barrington Tops National Park, Williams River Day Use Area, end of Blue Gum Track, 32°09′13.6″S 151°31′39.7″E, 355.1 m, 18 April 2010, DL Largent 9901(**holotype** DAR), sequences: HQ876546 (mtSSU), HQ876524 (LSU), HQ876543 (RBP2).

ETYMOLOGY — Derived from the Latin *parvulus* (= very little), referring to the very small pileus.

PILEUS 6 mm broad and 2 mm high, convex, entirely densely matted squamulose, overall orange-gray to grayish-orange (5B2-3) dull, even, dry, opaque and not hygrophanous; margin decurved, fringed, eroded and ± crenulate. TASTE AND ODOR indistinct. LAMELLAE 2 mm long, 1 mm deep, narrow, adnexed, orange-gray (5B2), distant; margin smooth but lighter in color; 1 lamellula between lamellae. STIPE 20 mm long, <1 mm broad, equal, dull, pruinose at the apex, glabrous elsewhere, orange-gray to grayish-orange (5B2), hollow and very fragile; stipe base not pruinose. BRUISING REACTION absent.

BASIDIOSPORES nodulose angular with 6–8(–9) angles, heterodiametric in profile view, 6-angled and isodiametric in polar view, 10.5–15.0 × 6.3–8.6 µm, (x_m = 13.2 ± 1.0 × 7.7 ± 0.6 µm; E = 1.35–2.04; Q = 1.72 ± 0.14; n = 29/1). BASIDIA 4-sterigmate, cylindro-clavate to clavate 32.5–40.4 × 10.8–14.8 µm, (x_m = 36.3 ± 2.6 × 12.8 ± 1.5 µm; E = 2.47–3.37; Q = 2.87 ± 0.29; n = 9/1). CHEILOCYSTIDIA solitary to scattered, versiform (aciculate, acuminate, obclavate, rostrate-ventricose), 36.0–88.6 × 14.8–21.3 µm. PLEUROCYSTIDIA absent. PSEUDOCYSTIDIA absent. ABORTED BASIDIA absent. PILEIPELLIS 100–200 µm deep, an entangled layer of hyphae composed of chains of thick walled cells, individual cells long and slender. PILEOCYSTIDIA cylindro-clavate, aciculate, aculeate to distinctly setiform 57.6–106.0 × 6.1–13.2 µm, (x_m = 74.8 ± 16.8 × 8.6 ± 2.2 µm; E = 7.01–16.00; Q = 9.04 ± 2.6; n = 11/1). STIPITIPELLIS a cutis from near the apex to the base of the stipe; apex overlooked because of stipe size. CAULOCYSTIDIA absent where studied. PIGMENTATION distinctly externally incrusted on the hyphae of the pileal stipe and lamellar trama; parietal and in the form of internal incrustations in the pileipellis.

ECOLOGY AND DISTRIBUTION: Solitary in soil amongst mosses on a bank cut in a wet sclerophyll subtropical gallery rainforest; known only from the type locality.

COMMENTS— *Pouzarella parvula* is differentiated by a small (6 × 2 mm) matted squamulose orange-gray pileus, adnexed lamellae, stipe pruinose at the apex and glabrous elsewhere, basidiospores averaging 13.2 × 7.7 µm, versiform

Plate 5 – *Pouzarella parvula* (DLL 9901, holotype). A: Field photo of basidiomata; B: Lamellae and lamellulae; C: Pileal surface and eroded pileal margin.

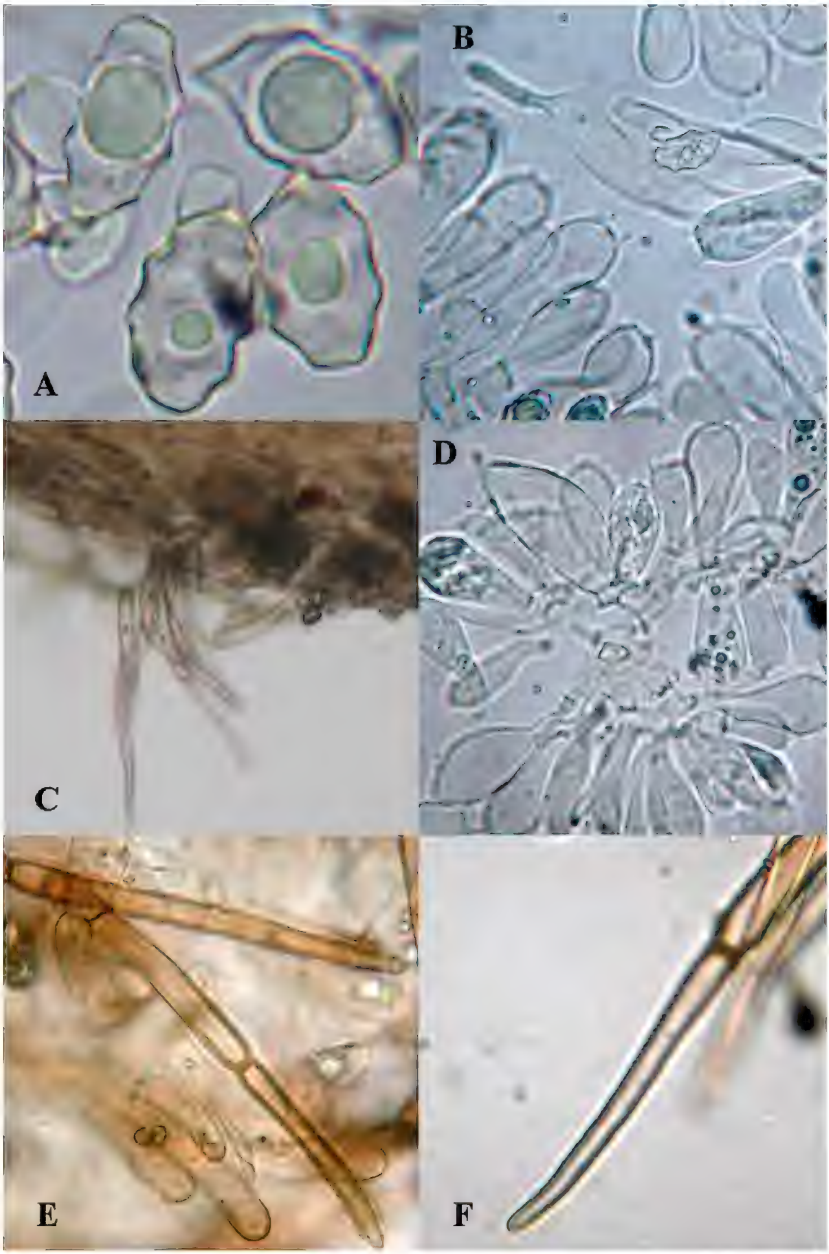

Plate 6 – *Pouzarella parvula* (DLL 9901, holotype). A: Basidiospores (1000×); B: Basidia and cheilocystidium (400×); C: Pileipellis near pileus margin (100×); D: Cheilocystidia (400×); E: Setiform pileocystidia (400×); F: Setiform pileocystidium (400×).

(some rostrate ventricose) cheilocystidia, entangled pileipellis with setiform pileocystidia, and lack of aborted basidia and caulocystidia.

Pouzarella minuta (E. Horak) E. Horak from New Zealand (Horak 2008), *Pouzaromyces napaliensis* Desjardin & T.J. Baroni from the Hawaiian Islands, and *Entoloma testaceostrigosum* Manim. & Noordel. from India have nearly the same size pileus as *P. parvula*. *Pouzarella minuta* differs by a smaller (2–4 × 1 mm) stipe densely covered with brownish or white fibrils and much larger basidiospores (16–20 × 10–12 µm) (Horak 1980, 2008). *Entoloma testaceostrigosum* and *Pouzaromyces napaliensis* both possess setiform pileocystidia and caulocystidia and lack cheilocystidia (Desjardin & Baroni 1991, Manimohan et al. 2006).

See *P. pilocystidiata* for comparisons with Australian species with a resemblance to *P. parvula*.

4. ***Pouzarella pamiae*** Largent, **sp. nov.** PLATES 7–8

MYCOBANK MB 519570

Habitu Pouzarellae squamifoliae *similis, sed coloribus basidiomatum fulvis, pileo latiore usque ad 3–8 mm, stipite minore, 12–25 × 0.5–0.75 mm atque contusis atro-fuscis differt.*

TYPE — AUSTRALIA, Queensland, Cook Region, Mossman Gorge National Park, 16°28′17.6″S 145°19′51.7″E, 84.4 m, 18 March 2010, DL Largent 9794 (**holotype** BRI, **isotype** CNS), sequences: HQ876539 (mtSSU), HQ876517 (LSU), HQ876496 (RBP2).

ETYMOLOGY —in honor of Pamela Largent, the collector and inspiration for the study of entolomatoid fungi in northeastern Queensland.

PILEUS 3–8 mm broad, 2–4.5 mm high, at first conic to conic-campanulate and densely erect squamulose-hispid over the entire surface expanding to convex and eventually convex-campanulate, remaining erect squamulose hispid on the center but becoming appressed squamulose from the margin first and then to near the center, at first entirely reddish-golden to brownish-orange (6-7C-D5-7) darkening to medium brown (6-7E-F5-6 teak brown to burnt umber) then dark brown (6-7E-F6-7 burnt umber to dark brown), with maturity remaining darker on the disc but lightening to yellowish-white to orangish-white (4-5A2-3), squamule tips lose color and separate showing the whitish-yellow background, not umbonate, opaque, dry and dull; margin decurved and fringed with squamule tips then some eroded as the tips collapse with age. TASTE indistinct. ODOR indistinct to pungent. LAMELLAE 3–4 mm long, 1.25–1.5 mm high, pale orange (6A3) then brownish (6E5 sunburn) with basidiospore maturity, narrow and then moderately broad, subdistant to nearly distant; no bruising reaction observed in the margin, fimbriate to serrulate and light-colored when lamellulae are mature. LAMELLULAE nearly non-existent with at most one lamellula between lamellae. STIPE 12–25 mm long, 0.5 mm broad at the apex, 0.75 mm broad at base, equal to slightly clavate, at first covered by a yellowish-white to orangish-white (4-6A2-3) dense layer of entangled

PLATE 7 – *Pouzarella pamiae* (DLL 9794, HOLOTYPE). A: Basidiomata, note densely hispid squamulose pileal surface and stipe base colored like pileus; B: Pileus with fringed pileal margin (left), lamellae (center) and young pileus surface (right).

fibrils, with maturity the fibrils darken to colors similar to those of the pileus, at times the consistency and color of the fibrils at the base are identical to those on the pileal surface, eventually the stipe surface becomes slightly roughened as the fibrils collapse onto the surface and then the stipe darkens to medium dark brown (6E6); the surface discolors to dark brown (near 6F6) when bruised; stipe base strigose or matted. Bruising reaction absent.

Basidiospores 7–9-angled, nodulose-angular, subisodiametric to heterodiametric in profile view, 5–6-angled and isodiametric in polar view, 10.0–15.9 (–17.3) × 7.1–11.2 µm (x_m = 13.07 ± 1.3 × 8.5 ± 0.8 µm; E = 1.18–1.85; Q = 1.53 ± 0.16; n = 82/2). Basidia 4-sterigmate, clavate, tapered, filled with a droplet, 31.8–48.3 × 11.5–15.3 µm (x_m = 42.3 ± 5.6 × 13.6 ± 1.2 µm; E = 2.17–3.78; Q = 3.11 ± 0.4; n = 11/1). Cheilocystidia abundant, broadly clavate to broadly acuminate, with a faint brownish cytoplasmic pigment, ± thick-walled, 47.1–77.6 × 10.1–26.2 µm and located as terminal cells arising from tramal hyphae with at least one subterminal cell inflated. Pleurocystidia absent. Pseudocystidia absent. aborted basidia inconspicuous or rare. Hyphae of the lamellar trama 79.3–240.3 × 5.9–15.3 µm. Pileipellis 111–551 µm deep, an entangled layer of long, rather slender hyphae with cells ± in chains, in young specimens entirely erect and trichodermoid, in older specimens trichodermoid only in the center, semi-erect towards the margin and repent at the margin; distinctly thick-walled in the apical 1–5 cells. Pileocystidia cylindric, cylindro-clavate, broadly clavate, or broadly obclavate, thick-walled and with parietal pigment, 26.0–78.7 × 6.6–13.0 µm (x_m = 46.8 × 9.8 µm; E = 2.87–7.52; Q = 4.8; n = 9/1). Hyphae of the pileal trama same size and pigmentation as lamellar trama. Stipitipellis similar to the pileipellis except with the addition of clusters of hymenial elements at the apex; Caulocystidia similar to the pileocystidia, 36.1–79.1 × 8.4–19.9 µm. Pigmentation externally incrusted in the trama, parietal and faintly externally incrusted in the pileipellis and stipitipellis.

Ecology and distribution: scattered in tan-colored soil beneath and protected by an overhanging boulder in a complex mesophyll vine forest on well-drained alluvium (Mossman Gorge National Park) or solitary in hard-packed soil (Danbulla National Park, Lake Euramoo).

Additional collections examined —**AUSTRALIA. Queensland**, Cook Region, Mossman Gorge National Park, 16°28′17.2″S 145°19′50.2″E, 86.6 m, 26 March 2010, DL Largent 9834 (mtSSU HQ876541, LSU HQ876519, RPB2 HQ876498); Danbulla National Park, Lake Euramoo Track, 17°09′42.3″S 145°37′45.6″E, 762.6 m, 21 March 2010, DL Largent 9808 (mtSSU HQ876540, LSU HQ876518, RPB2 HQ876497).

Comments— *Pouzarella pamiae* is diagnosed by its 3–8 mm broad densely squamulose-hispid pileus with brownish-orange squamules, long (12–25 mm) narrow stipe that bruises dark brown and has a base covered with squamules identical to those on the pileus, thick-walled acuminate to broadly clavate cheilocystidia, and basidiospores averaging 13.1 × 8.6 µm.

Plate 8 – *Pouzarella pamiae*. A: Basidiospores (1000×) (DLL 9808); B: Basidia (400×) (DLL 9794, holotype); C: Pileocystidia (100×) (DLL 9794); D: Thick-walled cheilocystidia with brownish pigment (400×) (DLL 9794); E: Cylindro-clavate pileocystidia (180×) (DLL 9794); F: Stipitipellis and chains of hyphae with terminal caulocystidia (100×) (DLL 9794).

Other species with a brownish-orange, densely squamulose-hispid pileus and stipe include *P. fulvolanata* (Berk. & Broome) Mazzer and *P. myoderma* (Berk. & Broome) T.J. Baroni (both from Ceylon), *P. squamifolia* (Murrill) Mazzer from Jamaica, *P. sepiaceobasalis* (E. Horak) T.J. Baroni from Argentina, and *P. ferreri* T.J. Baroni et al. from the Dominican Republic, Brazil, and Costa Rica.

Mazzer (1976) described *P. fulvolanata* with yellowish to tawny colors and basidiospores in the same size range (12–16 × 7–8 µm) as *P. pamiae*, but it can be differentiated by subpyriform cheilocystidia and larger pileus (13 mm broad) and stipe (32 mm × 2 mm). Noting their similar stature, coloration, and pileus and stipe surfaces, Mazzer (1976) differentiated *P. fulvolanata* and *P. squamifolia* based on distribution and stipe color: *P. squamifolia,* described from Jamaica, has a pallid stipe with ferruginous hairs whereas *P. fulvolanata,* described from Ceylon, has tawny hairs on the stipe.

The basidiospores, basidia, cheilocystidia, pileipellis, pileocystidia, stipitipellis and caulocystidia of *P. pamiae* are identical to those described for the Jamaican holotype of *P. squamifolia* (Mazzer 1976, Baroni et al. 2008). However, *P. squamifolia* is differentiated by ferruginous colors (tawny in *P. pamiae*), wider (10 mm broad) pileus, larger (40 × 1.5 mm) stipe, and the absence of a bruising reaction.

Pouzarella sepiaceobasalis is differentiated from *P. pamiae* by the longer basidiospores (15–19 µm) and longer stipe (60 mm) with a base that gradually becomes blue-black to black (Baroni et al. 2008).

Pouzarella ferreri is separated by a more reddish coloration, longer (≤60 mm) stipe, larger (~16.2 × 10.7 µm) basidiospores, globose to sphaeropedunculate cheilocystidia, and black staining reactions (Baroni et al. 2008).

On the basis of type studies, Pegler (1977, 1986) synonymised *A. fulvolanatus* Berk. & Broome under *A. myodermus* Berk. & Broome, for which he reported basidiospore measurements of 9–13(–15) × 5.5–7.0 µm (average 10.5 ± 1.0 × 6.5 ± 0.34 µm). He could not verify the presence of cheilocystidia, commenting, "Lamella-edge not revived." Based on further type studies, Horak (1980) synonymised both *A. myodermus* and *A. fulvolanatus* under *Agaricus lasius*, for which he reported basidiospores measuring 10.5–13.5 × 6–8 µm, cheilocystidia absent, and robust basidiomata with ≤20 mm broad pilei and 15–50 × 1–3 mm stipes. Therefore, *P. pamiae* is differentiated from both *Pouzaromyces myodermus* sensu Pegler (1977) and *Pouzaromyces lasius* sensu Horak (1980) by larger basidiospores, abundant clavate to acuminate cheilocystidia, and a smaller stature.

Australian taxa related to *Pouzarella pamiae*

The ML phylogeny weakly (BS <70) supports the clade including *P. pamiae* and *P. farinosa*. Both species possess ± thick-walled pigmented napiform to

clavate cheilocystidia and versiform pileocystidia. The two species are easily distinguished by basidiospore size (~15.5 × 10.0 µm in *P. farinosa*) and coloration (orange-gray squamules on a grayish-brown background in *P. farinosa*).

5. ***Pouzarella farinosa*** Largent & Skye Moore, **sp. nov.** PLATES 9–10

MYCOBANK MB 519571

Habitu Entolomate dysthali *(subg.* Pouzarellae*) similis, sed sapore farinaceo, squamulis pilei aurantiaco-griseis, cheilocystidiis latioribus, 21.1–51.1 × 16.7–30.9 um differt.*

TYPE — Australia, New South Wales, Cook Region, Central Hunter District, Barrington Tops National Park, Pool of Reflections Track, 32°08′15.8″S 151°30′38.2″E, 727.6 m, 22 April 2010, DL Largent 9934 (**holotype** DAR), sequences: HQ876538 (mtSSU), HQ876516 (LSU), HQ876495 (RBP2).

ETYMOLOGY — derived from the Latin *farinosus* (= mealy), referring to the mealy or farinaceous taste.

PILEUS 8–14 mm broad, 2–6 mm high, convex to broadly convex, in some with a slight, broad umbo, at first entirely squamulose-hispid with densely packed upright tufts of orange-gray (5B2) squamules on a grayish-brown (5E3) background, the overall color light grayish-brown (5C-D3), upon expansion remaining so in or near the center, becoming appressed squamulose and then appressed fibrillose towards the margin as the squamules get pressed onto the surface, dry, dull, opaque, not hygrophanous but becoming striate with age; margin decurved then slightly uplifted, striate with age, the striae grayish-brown (5D-E3). TASTE slightly to distinctly farinaceous. ODOR indistinct. LAMELLAE 3–6 mm long, 1–2 mm high, narrow, adnate with a distinct subdecurrent tooth, subdistant to distant, at first brownish-gray (5C3) then orange-white (6A2) with maturation of the basidiospores; margin whitish, smooth when very young eventually becoming minutely serrulate to fimbriate because of cystidia. STIPE 30–54 mm long, 1–1.25 mm broad, equal, fragile, dull, at first entirely orange-gray (5B2) and covered with dense visible tangled masses of fibrils and squamules that are more abundant towards the basal part of the stipe, with maturity and handling, the overall color grayish-brown (6C3-4) with areas darkening to dark brown (5F6) with age as the fibrils and squamules become appressed to the stipe surface. BRUISING REACTION absent.

BASIDIOSPORES 6–8-angled and nodulose angular in profile view, 6-angled in polar view, 12.0–19.8 × 7.9–11.9 µm (x_m = 15.5 ± 1.8 × 10.0 ± 0.84 µm; E = 1.2–1.8; Q = 1.56 ± 0.14 (heterodiametric); n = 63/2). BASIDIA 4-sterigmate, broadly clavate to clavate, tapered, 4-sterigmate with the sterigma long, slender and nearly needle-like with large droplets, 40.6–53.1 × 13.6–20.3 µm (x_m = 47.2 ± 3.5 × 16.3 ± 1.7 µm; E = 2.29–3.49; Q = 2.29 ± 0.34; n = 18/2). CHEILOCYSTIDIA abundant and forming a sterile layer mostly near the pileal margin, nearly globose, vesiculate, or nearly napiform, ± thick-walled, 25.1–51.7 × 16.7–30.9

Plate 9 – *Pouzarella farinosa.*
A: Basidiomata and stipe surface (DLL 9934, holotype); B: Pileus surface (DLL 9900).

µm, (x_m = 37.3 ± 8.2 × 23.0 ± 4.3 µm; E = 1.14–2.61; Q = 1.65 ± 0.40; n = 14/1). Pleurocystidia absent. Pseudocystidia absent. Aborted Basidia absent. Hyphae of the lamellar trama subparallel with relatively short cells, narrow and slender but more broad than those in the pileal trama, 4.4–87.4 × 3.6–10.7 µm. Pileipellis a densely entangled layer of hyphae in chains of 4–6 cells, ± palisade and erect in the center and near the center, semi-erect near the center and repent near the margin. Pileocystidia relatively short, clavate, broadly clavate or nearly napiform on or near the center, 21.4–54.1 × 8.6–20.4 µm (x_m = 42.4 ± 11.5 × 14.2 ± 4.3 µm; E = 1.35–4.87; Q = 3.24 ± 1.24; n = 9/1). Hyphae of the pileus trama similar to but more narrow than those in the lamellar trama. Stipitipellis 76–233 µm, similar to the pileipellis, composed of abundant clusters of hyphae in 5–6-celled chains. Caulocystidia clavate, cylindro-clavate, narrow to broadly aculeate, or even acuminate, longer than the pileocystidia, 32.4–76.6 × 9.1–20.6 µm, (x_m = 55.8 ± 14.4 × 14.3 ± 3.0 µm; E = 1.57–8.13; Q = 4.17 ± 1.74; n = 14/2). Oleiferous hyphae absent. Lipoid bodies absent in the trama; large globules present in the basidia. Pigmentation strongly incrusted on the pileal, lamellar and stipe trama; parietal and internally incrusted in the stipitipellis and pileipellis.

Ecology and distribution: Solitary to gregarious in soil, often amongst rocks and ferns in warm temperate and subtropical gallery rainforests, Central Hunter District, New South Wales, Australia.

Additional collections examined — **AUSTRALIA. New South Wales,** Cook Region, Barrington Tops National Park, Williams Day Use Area, end of Blue Gum Loop Track, 32°09′03.3″S 151°31′28.2″E, 366.9 m, 18 April 2010, DL Largent 9900 (mtSSU HQ876537, LSU HQ876515, RPB2 HQ876494).

Comments— *Pouzarella farinosa* is diagnosed by an entirely squamulose-hispid pileus covered by densely packed upright tufts of orange -gray squamules, basidiospores measuring 12.0–19.8 × 7.9–11.9 µm, nearly globose, vesiculate, or nearly napiform cheilocystidia, and the lack of aborted basidia.

The new species resembles *Entoloma dysthales* (Peck) Sacc. f. *dysthales* in basidiospore size, cheilocystidia, and overall stature and coloration. However, *E. dysthales* lacks the orange-gray colors of the *P. farinosa* squamules and possesses a mild (not farinaceous) taste and odor, abundant aborted basidia, and longer, narrower (21–82 × 8–21 µm) cheilocystidia. Our collections are similar to Gates 2265, the proposed holotype for a new species to be described in a forthcoming book on the entolomatoid fungi in Tasmania (Gates pers. comm.), but differs in the absence of aborted basidia, cheilocystidial shape (clavate to broadly clavate or obovoid in Gates 2265), and farinaceous taste.

See *P. pamiae* for comparisons with taxa closely related to *P. farinosa*.

Plate 10 – *Pouzarella farinosa*. A: Basidiospores (1000×) (DLL 9900); B: Pileipellis (400×) (DLL 9934, holotype); C: Basidia (400×) (DLL 9900); D: Thick-walled cheilocystidia with brownish pigment (400×) (DLL 9934); E: Pileocystidia (400×) (DLL 9900); F: Acuminate caulocystidia (400×) (DLL 9934).

6. ***Pouzarella setiformis*** Largent & Abell-Davis, **sp. nov.** PLATES 11–12

MYCOBANK MB 519586

Habitu Pouzaromyceti transito *similis, sed pileo juventute hispido squamuloso, squamulis primo pallido-coloratis tum cito atro-griseo-brunneo, postremo aetate pallide vel mediocriter brunneolo-griseo decolorantibus, peileocystidiis caulocystidiisque multicellularibus setiformibus differt.*

TYPE — Australia, Queensland, Cook Region, Danbulla National Park, Lake Euramoo Track, 17°09′41.1″S 145°37′46.9″E, 714.5 m, 21 March 2010, DL Largent 9809 (**holotype** BRI, **isotype** CNS), sequences: HQ876547 (mtSSU), HQ876525 (LSU), HQ876503 (RBP2).

ETYMOLOGY — derived from the Latin *seta* (= bristle) + *forma* (= shape), for setiform pileocystidia and caulocystidia.

PILEUS 8.5–12.5 mm broad, 3–5.5 mm high, convex then broadly convex, scurfy with ± erect light colored (6C3) brownish-orange ± hispid squamules on the disc, tomentulose to tomentose elsewhere, overall dark grayish-brown (6-7E-F2-3) fading to light or medium brownish-gray (6D3) with age, dull, opaque, dry, even when young becoming ± sulcate and striate with age, not hygrophanous; margin decurved then plane, minutely hispid. TASTE and ODOR indistinct. LAMELLAE 3–5.5 mm long, 1–2 mm high, narrow to moderately broad, adnexed to adnate, subdistant to distant, dark brownish-gray to dark grayish-brown at first (7-8E-F2-3); margin smooth and concolorous. LAMELLULAE 1–3 (2 short, 1 medium long) between lamellae. STIPE 15–25 mm long, 0.75–1.0 mm broad, equal, stiff but hollow, fragile and breaks easily, with abundant stiff, light brownish-gray (6D3 or 7C3) ± tomentulose pointed hairs and squamules at the apex, pruinose to fibrillose dark brownish-gray (6-7E-F3); elsewhere, dull, suggestively strigose at the base. BRUISING REACTION absent.

BASIDIOSPORES nodulose-angular with 6–8 angles, heterodiametric in profile view, isodiametric and 6-angled in polar view, 8.2–13.9 × 4.8–7.9 µm (x_m = 10.7 ± 1.0 × 6.3 ± 0.7 µm; E = 1.4–2.5; Q = 1.73 ± 0.24 (heterodiametric); n = 56/2). BASIDIA 25.7–32.9 × 8.5–12.2 µm; E = 2.11–3.85; Q = 2.95. CHEILOCYSTIDIA absent. PSEUDOCYSTIDIA absent. ABORTED BASIDIA often with dark brown contents in 3% KOH, aborted sterigma and/or aborted basidiospores often present, 26.2–41.0 × 6.8–10.7 µm. Subhymenium composed of thin and short hyphae. HYPHAE OF LAMELLAR TRAMA long and broad, 58.3–253.7 × 3.1–26.6 µm. PILEIPELLIS a dense compactly entangled chain of 3–5 cells, 55–144 µm deep in the center and towards the margin, at the margin with tufts of hyphae, 93–150 µm deep. PILEOCYSTIDIA cylindro-clavate in the center to near the margin, 23.9–64.9 × 8.8–17.4 µm (x_m = 40.5 × 12.8 µm; E = 2.20–4.07; Q = 3.13; n = 9/1), at the margin thick-walled and setiform, 42.4–69.9 × 4.5–5.7 µm. STIPITIPELLIS at the apex with tufts of heavily incrusted hyphae, 74–190 µm deep, entangled hyphae between the tufts. CAULOCYSTIDIA aculeate to aciculate, clear and ± setiform when in abundant tufts, 47.0–121.0 ×

Plate 11 – *Pouzarella setiformis* (DLL 9809, holotype). A: Basidiomata; B: Pileipellis (100×); C: Basidiospores (1000×); D: Basidia and pseudocystidia (400×).

Plate 12 – *Pouzarella setiformis*. A: Pileocystidia with parietal pigmentation (400×) (DLL 9809, holotype); B: Multicellular setiform pileocystidia (400×) (DLL 9809); C: External incrustations on tramal hyphae of pileus (400×) (DLL 9810); D: Multicellular setiforma caulocystidia (100×) (DLL 9809).

4.8–7.9 µm; E = 8.44–17.50; Q = 10.89; clear and acuminate when not in tufts, 17.4–61.3 × 5.5–11.6 µm; E = 1.93–6.15; Q = 3.13. Pigmentation heavily incrusted on the outer walls of the tramal hyphae of the stipe, pileus and lamellae; parietal and heavily incrusted on inner and outer cell walls of the pileipellis and stipitipellis excluding the pileocystidia and caulocystidia; thick-walled, not incrusted, ± suggestively parietal but mostly clear in the setiform pileocystidia and caulocystidia.

Ecology and distribution: Solitary or scattered in hard packed soil in a complex mesophyll vine forest; northeastern Queensland.

Additional collections examined — **AUSTRALIA. Queensland**, Cook Region, Danbulla National Park, Lake Euramoo track, 17°09′41.6″S 145°37′40.7″E, 714.5 m, 21 March 2010, DL Largent 9810 (mtSSU HQ876548, LSU HQ876526, RPB2 HQ876504).

Comments— *Pouzarella setiformis* is diagnosed by a pileus that is hispid-squamulose on disc and elsewhere tomentulose to tomentose with hairs that are initially light colored but quickly become dark gray brown before fading to light or medium brownish-gray with age and a young even margin that becomes ± sulcate and striate with age, nodulose-angular, heterodiametric basidiospores averaging 10.7 × 6.3 µm, the absence of cheilocystidia, and the presence of aborted basidia, setiform pileocystidia and caulocystidia, and encrusted pileipellis and stipitipellis hyphae.

Pouzaromyces napaliensis from the Hawaiian Islands, *P. transitus* E. Horak from Papua New Guinea, and *Entoloma testaceostrigosum* from India have nodulose-angular heterodiametric basidiospores and setiform caulocystidia and lack cheilocystidia, features common to *P. setiformis*. *Pouzaromyces napaliensis* is distinguished by its slightly larger (11.2–15.4 × 7–9.6 µm) basidiospores, uniformly beige pileus and lamellae, smaller size (2–5 mm broad pileus, 3–4 mm long stipe), and weakly incrusted tramal hyphae (Desjardin & Baroni 1991). *Pouzaromyces transitus* differs in its black to soot-brown basidiomata and pileus hairs and single-celled setiform caulocystidia (Horak 1980). *Entoloma testaceostrigosum* is separated by its larger (12.5–17.0 × 7–10 µm) basidiospores and absence of encrusted pileipellis and stipitipellis hyphae (Manimohan et al. 2006).

Australian taxa related to *Pouzarella setiformis*

Pouzarella setiformis, P. albostrigosa, *P. fusca*, *P. lasia* and *P. debilis* form a monophyletic group that share a brown squamulose hispid pileus, stipe surface composed of a layer of entangled brownish fibrils, pileipellis composed of a chain of cells with clavate to broadly clavate pileocystidia, more or less stipitipellis with occasional entirely clavate to broadly cylindro-clavate caulocystidia, basidiospores averaging 10.0–12.0 µm long, aborted basidia that become reddish-brown in 3% KOH, and absence of cheilocystidia.

Within this group *P. setiformis* differs from *P. debilis*, *P. fusca*, *P. albostrigosa,* and *P. lasia* by a stipe surface with abundant stiff, ± tomentulose pointed hairs that is pruinose to fibrillose except for the apex squamules and a stipitipellis and pileipellis with scattered to abundant setiform caulocystidia and pileocystidia (most evident at the margin). The other taxa in this clade (*P. debilis*, *P. fusca*, *P. albostrigosa* and *P. lasia*) possess instead a stipe surface that is densely wooly or covered with abundant loose fibrils to nearly glabrous and non-setiform cystidia.

Pouzarella debilis, P. fusca, and *P. albostrigosa* share a convex to broadly convex pileus, a relatively shorter (typically ≤20 mm) stipe, aborted basidia typically imbedded in the hymenium, and caulocystidia, pileocystidia, and many subterminal cells with strong parietal pigmentation comprising coarse bands and obvious internal incrustations. *Pouzarella fusca* produces a young

pileus that is opaque and black to dark gray brown, basidiospores averaging 10.0 × 7.3 µm, and caulocystidia of 2 types (some thick-walled and pigmented). *Pouzarella albostrigosa* has light colored squamules when young, basidiospores averaging 11.5 × 7.7 µm, and caulocystidia of 2 types (some thick-walled and pigmented). *Pouzarella debilis* combines light-colored squamules when young, basidiospores averaging 10.4 × 6.3 µm, and thin-walled ± colorless caulocystidia, similar in shape to the pileocystidia.

Lastly, *P. lasia* produces a conic to campanulate pileus (at least when young), a stouter ≤50 mm long stipe, aborted basidia that distinctly project beyond the hymenium, caulocystidia and pileocystidia with obscure bands and minute internal incrustations, and absence of setiform pileocystidia and caulocystidia.

7. ***Pouzarella debilis*** (Corner & E. Horak) Largent & Abell-Davis, **comb. nov.**

MycoBank MB 519572 Plates 13–14

≡ *Pouzaromyces debilis* Corner & E. Horak, Beih. Nova Hedwigia 65: 42 (1980).

Pileus 5 mm broad, 4 mm high, convex, at first entirely opaque and hispid-squamulose with the squamules off-white (5A-B1-2) and erect in the center, suberect elsewhere, base color of the center grayish-brown (6F3-4) and elsewhere slightly lighter grayish-brown (6E-F3-4) when faded remaining so on the disc but becoming appressed squamulose and striate to the margin, elsewhere with the base color light grayish-brown (near 6C3-4) and the striae darker grayish-brown (6E3-4), dry, dull, not hygrophanous, even; margin decurved, minutely fringed and crenulate at the very edge of the margin. Taste and Odor unknown. Lamellae 7.5 mm long, uncinate with a short decurrent tooth, close to subdistant, narrow (1 mm deep); margin smooth and concolorous. Lamellulae very short, commonly 1, infrequently 3 (all short) between 2 lamellae. Stipe 15 mm long, 0.5 mm broad, equal, on the surface with loosely entangled fibrils or scattered pruina, obvious at the apex and base, inconspicuous elsewhere, surface fibrils similarly colored to those of the pileus, base color grayish-orange (6B-C3-4) and upon handling the base bruises a grayish-brown (6E3-4), hollow and fragile because of size. Bruising reaction absent.

Basidiospores nodulose-angular with 6–8 angles, heterodiametric in profile view, isodiametric and 6-angled in polar view, 8.7–12.0 × 5.0–7.4 µm, (x_m = 10.44 ± 0.8 × 6.3 ± 0.6 µm; E = 1.36–2.27; Q = 1.67 ± 0.22; n = 29/1). Basidia 4-sterigmate, clavate, tapered, 27.6–36.5 × 8.9–12.8 µm, (x_m = 31.8 ± 2.7 × 11.1 ± 1.2 µm; E = 2.33–3.49; Q = 2.88 ± 0; n = 10/1). Cheilocystidia absent. Pseudocystidia absent. Aborted basidia abundant on the gill edge and face, colored reddish-brown in 3% KOH, often with 1–2 sterigma and aborted basidiospores, slightly projected beyond the hymenium, 33.6–46.8 × 7.4–11.6 µm. Hyphae of lamellar trama subparallel, moderately long,

Plate 13 – *Pouzarella debilis* (DLL 9784). A–B: Basidiomata.

narrow to fairly wide, 184.9396.8 × 6.2–30.8 µm. Pileipellis with scattered, entangled hyphae composed of chains of 3–5 cells, subpellis without inflated cells, hyphae similar to pileal trama. Pileocystidia broadly cylindro-clavate 35.1–58.4 × 9.7–20.0 µm (E = 2.49–4.10; Q = 3.2). Stipitipellis with scattered areas of clustered hyphae similar to those of the pileipellis, 29–103 µm long, a cutis between the clusters. Caulocystidia similar to pileocystidia, 20.7–54.4 × 7.0–16.5 µm (x_m = 34.9 ± 10.6 × 11.6 ± 2.7 µm; E = 1.82–6.41; Q = 3.14 ± 1.31; n = 16/1). Pigmentation moderately externally incrusted in the trama of the pileus, stipe and lamellae; parietal in the form of minute to moderate internal incrustations in the hyphae of the stipitipellis and pileipellis.

Ecology and distribution: Solitary in dark crumbly soil on an open hillside in complex mesophyll vine forest of cloudy to moist highlands on granite; Mt. Hypipamee National Park. Previously reported from Papua New Guinea and the Solomon Islands.

Plate 14 – *Pouzarella debilis* (DLL 9784). A: Basidiospores (1000×); B: Basidium (black arrow), pseudocystidium (white arrow) (400×); C: Caulocystidia (400×); D: Pileipellis hyphae with clavate to broadly cylindro-clavate pileocystidia (400×).

Collections examined — **AUSTRALIA. Queensland,** Cook Region Mt. Hypipamee National Park, Falls Loop Track, 17°25′36.9″S 145°29′07.9″E, 714.5 m, 16 March 2010, DL Largent 9784 (holotype BRI, isotype CNS; mtSSU HQ876550, LSU HQ876528, RPB2 HQ876506).

Comments— *Pouzarella debilis* is diagnosed by small fragile basidiomata (pileus 5 mm broad, stipe 15 × 0.5 mm), a hispid-squamulose pileus at first with off-white squamules that are erect on the disc and elsewhere suberect over a medium to dark grayish-brown base color and in age becoming striate from the margin inwards and fading to light grayish-brown, aborted basidia that project slightly beyond the hymenium, absence of cheilocystidia, caulocystidia resembling the pileocystidia, and basidiospores averaging 10.4 × 6.3 µm, (Q = 1.67). The macro- and micro-morphological features of the Australian

collection *P. debilis* are nearly identical with those described for *Pouzaromyces debilis* from Papua New Guinea and the Solomon Islands (Horak 1980).

Pouzarella domingensis T.J. Baroni from the Dominican Republic and *P. dunstervillei* (Dennis) Mazzer have moderate-sized basidiospores and lack cheilocystidia, features also noted for *P. debilis*. *Pouzarella domingensis* differs in the lack of light-colored squamules in young pilei, inflated subpellis hyphae, and larger basidiospores averaging 11.0 × 7.6 µm (Baroni et al. 2008), while *P. dunstervillei* (which also lacks light-colored squamules in young pilei) has a nearly glabrous pileus and stipe (Mazzer 1976). *Pouzarella pulverea* (Rea) Mazzer from Great Britain and the Netherlands, *Entoloma romagnesii* Noordel. from France, and *E. lomapadum* Manim. et al. from India also have similarly sized basidiospores, but all three lack light-colored squamules in the young pilei and possess thin walled cheilocystidia (Mazzer 1976, Manimohan et al. 1995, Noordeloos 1979, 2004).

See *P. setiformis* for comparisons with other Australian entolomatoid fungi similar to *P. debilis*.

8. ***Pouzarella fusca*** Largent & Abell-Davis, **sp. nov.** PLATES 15–16

MYCOBANK MB 519573

Habitu Entolomate romagnesii *(subg.* Pouzarellae*) similis, sed pileo infuscate atro-brunneo, pigmento extrorsum in hyphis ad partes pileipellis stipitipellisque pertinentibus incrustato, pigmentatione perietali pileocystidiorum cauocystidiorumque, cheilocystidiis nullis differt.*

TYPE — Australia. Queensland, Cook Region, Dinden National Park, 17°02′14″S 145°36′4″E, 727.6 m, 24 February 2009, DL Largent 9623 (**holotype** BRI, **isotype** CNS), sequences: HQ876549 (mtSSU), HQ876527 (LSU), HQ876505) (RBP2).

ETYMOLOGY — derived from Latin *fuscus* (= dark brown), referring to the dark blackish-brown coloration.

PILEUS 3.5–10 mm broad, 1.5–3.0 mm high, consistently convex, minutely but entirely squamulose, erect in the center, imbricate squamulose elsewhere, becoming appressed squamulose at the very margin, black to a very dark gray brown (not matching any color to Kornerup & Wanscher (1978), fading to dark gray-brown (6F3) except a medium gray-brown (6E-F3) on the margin, dull, opaque, not hygrophanous, not striate; trama dark gray to black, less than 0.5 mm thick above the stipe; margin slightly incurved to decurved, entire. TASTE mild. ODOR somewhat pungent. LAMELLAE 2.5–3.5 mm long, 0.5–1.0 mm high, concolorous with the pileus, adnate, subdistant and narrow; margin smooth and concolorous. LAMELLULAE 3 (2 short, 1 medium) between lamellae. STIPE 8–17 mm long, 0.8–2.0 mm broad, equal, dark gray (6F1), minutely and entirely squamulose to covered with loose fibrils; basal tomentum scarce, strigose base present. BRUISING REACTION absent.

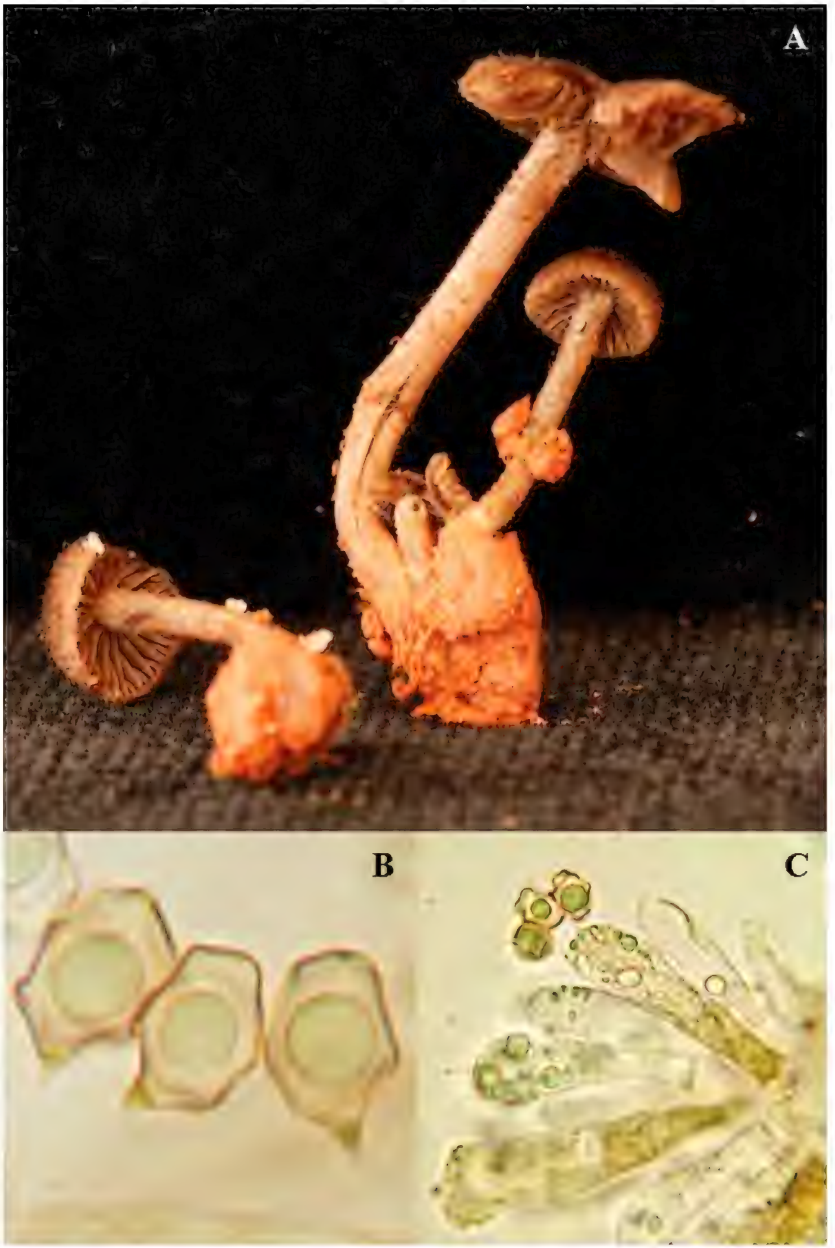

Plate 15 – *Pouzarella fusca* (DLL 9623, holotype).
A: Basidiomata; B: Basidiospores (1000×); C: Basidia with basidiospores (400×).

Basidiospores distinctly angular to slightly nodulose, in profile view typically 6-angled, often 7–8-angled, nearly or definitely heterodiametric, 8.6–11.5 × 6.1–8.7 µm (x_m = 10.0 ± 0.8 × 7.3 ± 6 µm; E = 1.22–1.54; Q = 1.38 ± 0.09 (heterodiametric); n = 28/1). Basidia 4-sterigmate, sterigma rather long, 4.2–5.5 µm; clavate, origin unusual for entolomatoid fungi, originating from a basal cell in the subhymenium, 33.9–47.2 × 8.9–12.6 µm (E = 2.8–4.7; Q = 3.83); base 3.0–6.5 µm. Pleurocystidia absent. Pseudocystidia absent. aborted basidia abundant, but scattered on the lamellae, clavate, often with aborted sterigma, not projecting beyond the hymenium and filled with opaque contents that stain reddish-brown in 3% KOH, 35.0–44.6 × 8.7–13 µm (x_m = 40.9 × 11.0 µm; E = 3.1–4.6; Q = 3.78; n = 7/1). Hyphae of the lamellar trama rather short, 29.6–179.0 × 4.7–11.0 µm. Pileipellis 240–400 µm deep, composed of chains of cells, erect or suberect, on or near the disc and towards the margin, becoming repent at the margin. Pileocystidia clavate to broadly cylindric, 36.5–118.9 × 5.2–21.4 µm (E = 2.6–6.1; Q = 5.04). Hyphae of the pileal trama rather short, 45.5–105.4 × 2.1–8.0 µm. Stipitipellis similar to the pileipellis and composed of solitary caulocystidia. Caulocystidia of two types; cylindro-clavate and the terminal cells comprised of a loosely entangled hyphae, 20.8–73.2 × 4.7–10.9 µm (E = 3.6–8.1; Q = 5.53), and clavate to broadly napiform and the terminal cells comprised of solitary hyphal walls and in clusters with the hyphae originating from a common base, 22.3–57.7 × 9.1–14.3 µm (E = 1.7–5.4; Q = 2.71). Hyphae of the stipe trama parallel to subparallel, 60.5–336.3 × 4.5–12.5 µm. Oleiferous hyphae rare in the trama of the stipe, pileus and lamellae. Lipoid bodies present in the basidia, absent in the aborted basidia and tramal hyphae of the pileus, lamellae and stipe. Pigmentation externally incrusting the hyphae of the basal portion of the pileipellis and stipitipellis and distinct and strong on the tramal hyphae of the lamellae, pileus and stipe; suggestively externally incrusting the outer hyphal and more obviously parietal on the inside the hyphal walls of the terminal 2–3 cells of the pileipellis and stipitipellis, including the pileocystidia and the caulocystidia.

Ecology and distribution: Scattered or connate in dark soil on high banks along road just above bridge at end of Davies Creek Road in a simple to complex notophyll vine forest, Dinden National Park.

Comments— *Pouzarella fusca* is diagnosed by the dark gray to dark blackish-brown to black coloration when young, a pileus that is initially squamulose on the disc and imbricate squamulose near the margin, only slightly nodulose

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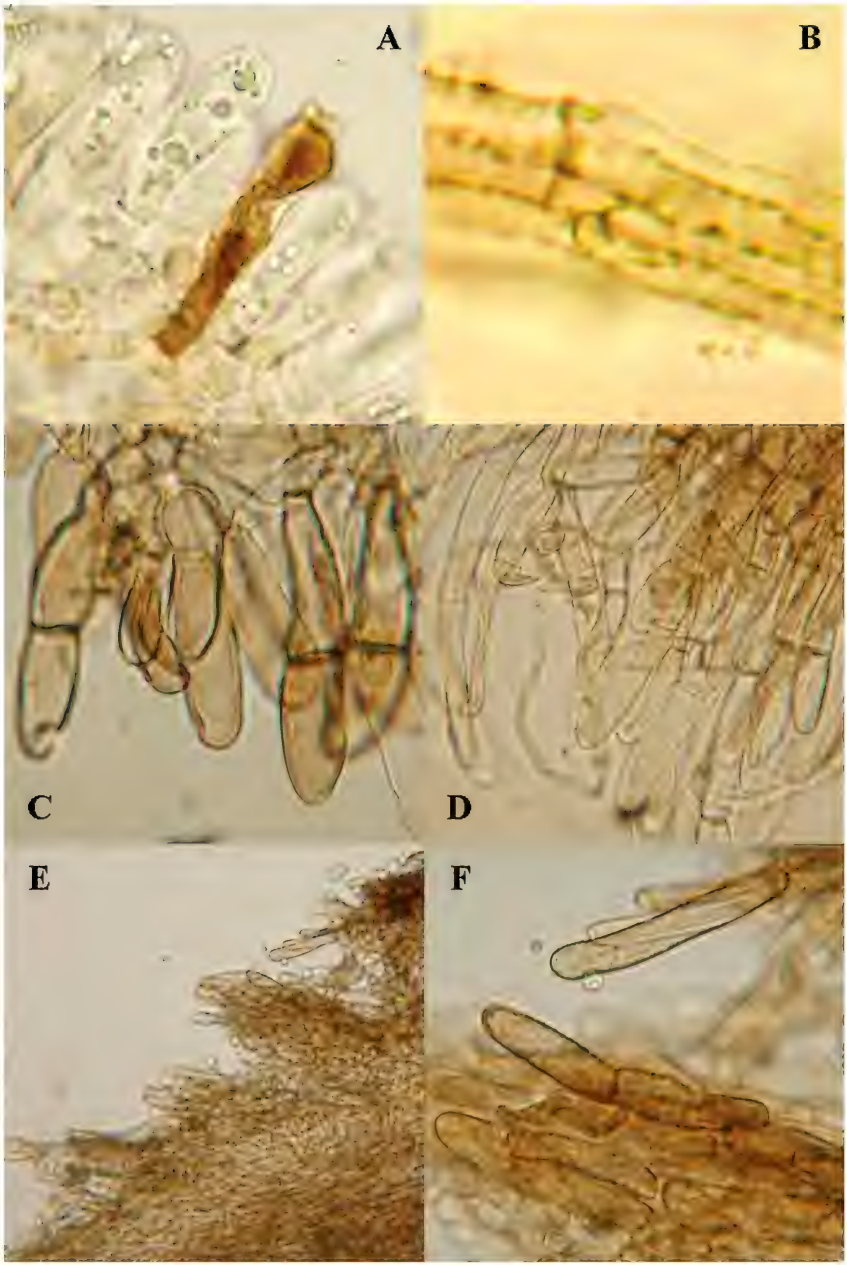
Plate 16 – *Pouzarella fusca* (DLL 9623, holotype). A: Pseudocystidium, origin from incrusted hyphae in subhymenium, basidium origin from non-incrusted hyphae (400×); B: Externally incrusted pigment on walls of lamellar trama (400×); C: bullet-shaped caulocystidia (400×);



D: cylindro-clavate caulocystidia (400×); E: Pileipellis near pileus center (100×); F: Cylindro-clavate to clavate pileocystidia (400×).

6–8 angled basidiospores averaging 10.0 × 7.3 µm, caulocystidia of two types (cylindro-clavate or napiform to clavate), and abundant aborted basidia.

The distinctive features of *P. fusca* are not found in any other *Pouzarella* species. *Entoloma romagnesii* described from France has similarly shaped and sized basidiospores but has a dark yellow-brown pileus and stipe, broadly ellipsoid to clavate cheilocystidia, and non-encrusted pileus and stipe hairs (Noordeloos 1979).

See *P. setiformis* for comparisons with other Australian entolomatoid fungi similar to *P. fusca*,.

9. ***Pouzarella albostrigosa*** Largent & Abell-Davis, **sp. nov.** PLATES 17–18

MYCOBANK MB 519587

Habitu Pouzarellae lasiae *similis, sed pileo convexo vel late convexo, base stipitis alba distincte strigosa, stipite minus robusto usque ad 22 mm longo, pseudocystidiis in hymenio inclusis, caulocystidiis pileocystidiisque pigmentatione parietali in parietibus internis e fasciis tumoribusque composita differt.*

TYPE — Australia, Queensland, Cook Region, Mt. Hypipamee National Park, Kauri Creek Track, 17°25′35.7″S 145°29′11.4″E, 964.7 m, 10 March 2009, DL Largent 9641 (**holotype** BRI, **isotype** CNS), sequences: HQ876557(mtSSU), HQ876535(LSU), HQ876513 (RBP2).

ETYMOLOGY — derived from the Latin *albus* (= white) + *strigosus* (= bristly), referring to the white stipe base.

PILEUS 3–10 mm broad, 0.8–2.5 mm high, convex to broadly convex, at first opaque, entirely hispid-squamulose with light colored squamules (between white and orange-white or reddish-white, 5-7A-B1-2) on a dark smoky brown to blackish-brown background (6-7F2-3), upon expanding and at maturity remaining hispid-squamulose on the disc, appressed squamulose to appressed fibrillose toward the margin and appressed fibrillose at the margin with an overall color of blackish-brown on the disc (close to but darker than 7F2) and slightly lighter elsewhere (close to but darker than 6-7F2-3) and becoming striate up to the center, eventually fading overall to dark grayish-brown (5F3) but remaining striate, dull, not hygrophanous and even; margin entire, decurved and opaque then striate. TASTE indistinct to suggestively farinaceous. ODOR indistinct. LAMELLAE 2.5–5.5 mm long, adnate to uncinate, subdistant to distant, narrow to moderately broad (1.0–1.8 mm high), dark gray to dark brownish-gray (7F2), and then appearing dark grayish-brown (5E-F4) and eventually light grayish-brown (5C-D3) after basidiospore maturation; margin smooth, somewhat lighter than the surface. STIPE 6–22 mm long, 0.8–1.0 mm broad at the apex, equal, covered with a dense layer of light colored fibrils or squamules on a dark brown (5-7E-F3-4) faintly fibrillose surface, solid at first, then hollow, basal tomentum absent to scarce; basal area pruinose just above white, distinctly strigose base. BRUISING REACTION stipe bruising dark

PLATE 17 – *Pouzarella albostrigosa* (DLL 9641, HOLOTYPE).
A: Basidiomata stature; B: Pileal surface; C: Lamellae and lamellulae.

gray brown when surface fibrils become appressed to the surface. BRUISING REACTION absent.

BASIDIOSPORES moderately nodulose-angular with 7–9 angles, heterodiametric in profile and side views, isodiametric and 6-angled in polar view, 9.1–15.6 × 6.7–9.2 µm (x_m = 11.5 ± 1.4 × 7.7 ± 0.6 µm; E = 1.27–2.16; Q = 1.49 ± 1.6; n = 57/3). BASIDIA 2–4 sterigmate, clavate and tapered, colorless in 3% KOH, 21.0–69.4 × 7.1–13.2 µm (x_m = 38.8 ± 14.0 × 9.7 ± 1.6 µm; E = 2.06–6.71; Q = 4.07 ± 1.50; n = 24/2). PLEUROCYSTIDIA absent. PSEUDOCYSTIDIA absent. ABORTED BASIDIA very abundant, nearly always with 2–4 sterigma and a few with aborted basidiospores, clavate and tapered, equal to but more often ⅓ to ¼ more narrow at the base than the basidia, embedded in the hymenium and not projecting beyond the basidia, often but not always, originating from the outer portions of the lamellar trama, dark reddish-brown in 3% KOH, 28.6–41.9 ×

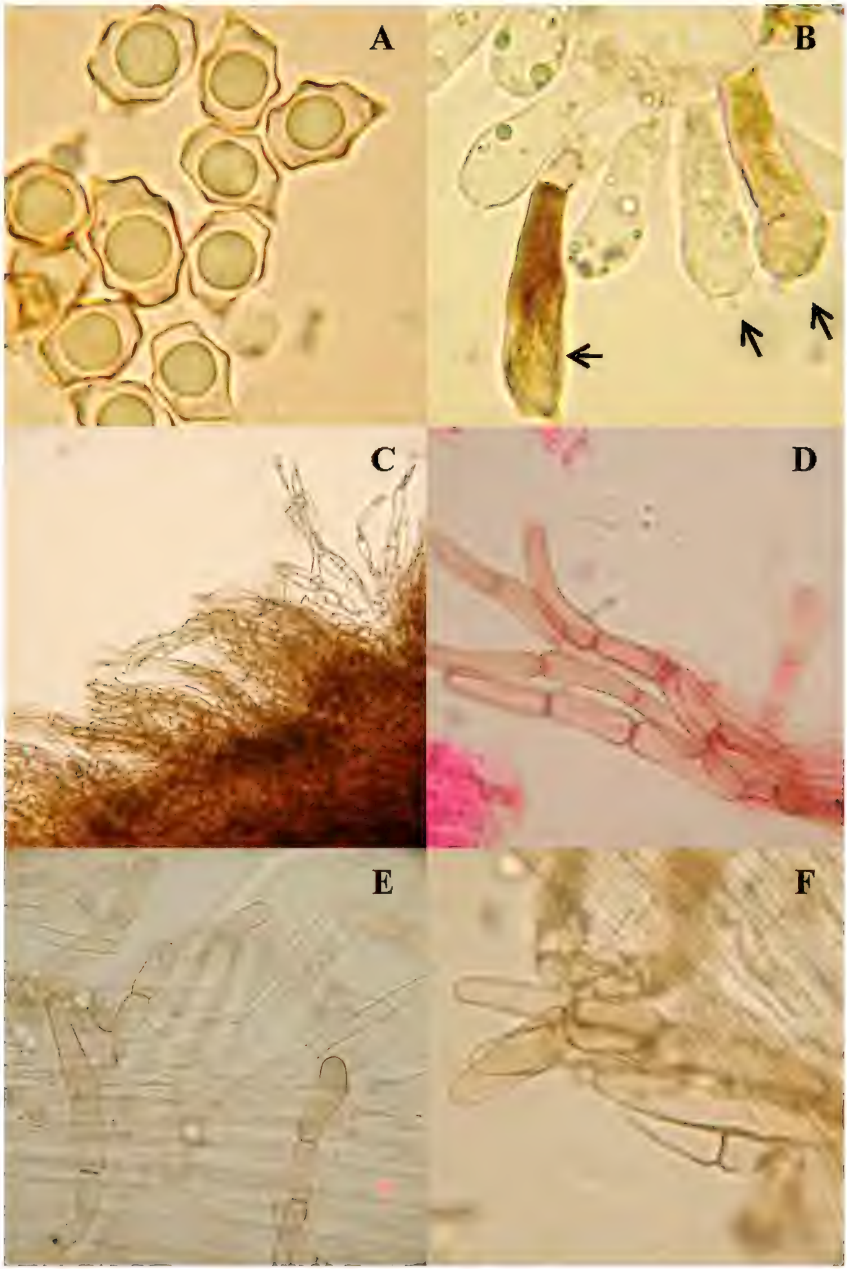


Plate 18 – *Pouzarella albostrigosa*. A: Basidiospores (1000×) (DLL 9641: holotype); B: Pseudocystidia (black arrows) (400×) (DLL 9641); C: Pileipellis in disc area (100×) (DLL 9663);

7.1–12.2 µm (E = 2.69–4.33; Q = 3.71 ± 0.48; n = 11/1). LAMELLAR TRAMAL HYPHAE did not revive well and was not measured. PILEIPELLIS up to 100–150 µm deep, composed of clusters (i.e. the squamules) of 5–10 laterally attached hyphae, each hypha with 5–10 cells; the clusters tapered with 5–10 hyphae laterally agglutinated at the base and 2–4 hyphae laterally agglutinated at the apex, erect on the disc, semi-erect towards the margin and nearly repent at the margin. PILEOCYSTIDIA typically narrowly cylindro-clavate, at times broadly cylindro-clavate, rarely clavate, 22.5–63.6 × 5.7–21.6 µm (x_m = 41.2 ± 9.1 × 11.1 ± 3.8 µm; E = 1.42–8.40; Q = 4.20 ± 1.80; n = 36/3) PILEAL TRAMAL HYPHAE did not revive well and was not measured. STIPITIPELLIS a cutis of pigmented hyphae between abundant scattered clusters of pigmented hyphae with clavate, broadly obclavate or broadly cylindro-clavate thick-walled; pigmented caulocystidia and scattered clusters of loosely entangled hyphae, 5–10 cells long, with cylindro-clavate to clavate, colorless caulocystidia. CAULOCYSTIDIA 21.0–69.4 × 7.9–13.2 µm (n = 24/3). STIPE TRAMAL HYPHAE parallel, 86–330 × 6–29 µm (n = 17/1). OLEIFEROUS HYPHAE rare to nearly absent, reddish-brown in 3% KOH. PIGMENTATION distinct, coarsely and externally incrusting the tramal hyphae of the stipe and most probably the pileus and lamellae; parietal and with bands and bumps on the inner walls of the pileipellis, stipitipellis, pileocystidia and some caulocystidia, externally incrusting the outer walls of the stipitipellis; uniformly cytoplasmic in some pileocystidia and caulocystidia.

ECOLOGY AND DISTRIBUTION: Solitary in dark crumbly soil on hillside with water pipe above a trail or in hard packed soil within a complex mesophyll vine forest of high rainfall; northeastern Queensland.

ADDITIONAL COLLECTIONS EXAMINED — **AUSTRALIA. QUEENSLAND**, Cook Region, Mt. Hypipamee National Park, 17°25′35.7″S 145°29′11.4″E, 964.7 m, 18 March 2009, DL Largent 9666; Danbulla National Park, Lake Euramoo Track, 17°09′42.3″S 145°37′45.6″E, 762.6 m, 18 March 2009, DL Largent 9663 (mtSSU HQ876558, LSU HQ876536, RPB2 HQ876514).

COMMENTS— *Pouzarella albostrigosa* typically has a convex pileus that is hispid-squamulose on the disc and imbricate squamulose elsewhere and initially covered with white to orange-gray or reddish-gray tipped squamules that become dark brownish-gray with age and exposure, a ≤22 long stipe covered by light-colored layer of entangled fibrils overlying longitudinally appressed dark brown fibrils and with distinct white strigose fibrils at the base, nodulose-angular basidiospores averaging 11.5 × 7.7 µm, absence of cheilocystidia, and the presence of aborted basidia embedded in the hymenium, pileocystidia and caulocystidia with internal parietal pigmentation in the form of bands and

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D: Pileocystidia (400×) (DLL 9641); E: Stipitipellis squash mount showing chains of cells and caulocystidia as terminal cells (400×) (DLL 9641); F: Stipitipellis periclinal section showing chains of cells with caulocystidia as terminal cells (400×) (DLL 9641).

bumps (some uniformly pigmented) and both thick-walled pigmented and thin-walled colorless caulocystidia.

See *Pouzarella setiformis* for comparisons with Australian taxa related to *P. albostrigosa*.

10. ***Pouzarella lasia*** (Berk. & Broome) Largent & Abell-Davis, **comb. nov.**

MycoBank MB 519574 Plates 19–20

≡ *Agaricus lasius* Berk. & Broome, J. Linn. Soc., Bot. London. 11: 539 (1871).

Pileus 4–10 mm broad, 2.5–7 mm high, conic to campanulate, at first hispid-squamulose on the disc and imbricate squamulose elsewhere, remaining so on the disc when mature but becoming appressed squamulose towards the margin and appressed fibrillose on the margin, the squamules entirely pallid to brownish-orange (5C3) at first, with age darkening from the base towards the tip, dark brownish-gray (5E-F3), eventually entirely dark brownish-gray (5-7F2-3) on the disc and a lighter brownish-gray elsewhere (5-7E2-3); marginal fibrils concolorous with nearby squamules dull, opaque and even at first, becoming striate when faded; margin decurved, entire. Taste and Odor indistinct. Lamellae 2–6 mm long, rarely adnexed to more commonly uncinate, subdistant, narrow to moderately broad (1–2 mm deep) when 2–6 mm long, when young entirely brownish-gray (5E-F3-4) and then appearing brownish-orange (5C3) with a lighter-colored margin with basidiospore maturation, margin smooth. lamellulae typically 3 between lamellae and in 2 tiers (2 short and 1 medium long). Stipe 11–40(–50) × 0.5–1.3 mm, equal, with a superficial, orange-gray (5B2-3) to brownish-orange (5C3) layer of entangled fibrils overlying longitudinally appressed, grayish-brown (5D-E3-4) fibrils, solid becoming hollow with age; basal tomentum scarce to absent; stipe base pallid and obscurely strigose. Bruising reaction observed in the stipe (7F2-3) but not the strigose base, darkening upwards.

Basidiospores nodulose-angular with 7–9 angles in profile and side views, heterodiametric, 8.9–14.5 × 5.1–8.7 µm (x_m = 11.5 ± 1.2 × 7.2 ± 0.6 µm; E = 1.29–1.99; Q = 1.61 ± 0.14; n = 99/4). Basidia 2–4 sterigmate, clavate but hardly tapered, colorless in 3% KOH, 34.6–42.7 × 7.7–11.8 (widest point) × 5.2–7.9(base) µm (E = 2–96–5.28; Q = 4.02). Pleurocystidia absent. Pseudocystidia absent. Aborted basidia abundant, nearly always with 2–4 sterigma and a few with aborted basidiospores, clavate and tapered to a narrow end, often embedded in the hymenium but a few projecting up to 17 µm beyond the basidia, often but not always, originating from the outer portions of the lamellar trama, dark reddish-brown in 3% KOH, 36.2–59.0 × 7.1–11.2(widest point) × 2.8–5.2(base) µm (E = 4.76–5.58; Q = 5.02). Lamellar tramal hyphae parallel to subparallel, 22.8–215.2 × 4.0–25.7 µm; narrow hyphae abundant, broad hyphae rare to uncommon; subhymenium indistinct.

Plate 19 – *Pouzarella lasia* (DLL 9662).
A: Basidiomata stature; B: Pileus surface; C: stipe surface.

Pileal tramal hyphae 56.0–162.3 × 3.0–13.5 µm. Pileipellis up to 240 µm deep, composed of clusters (squamules) of 5–10 laterally attached hyphae, each hypha with 10–20 cells that have parietal pigmentation; the clusters tapered with 5–10 hyphae at the base and 2–4 hyphae at the apex, erect on the disc, semi-erect towards the margin and nearly repent at the margin. Pileocystidia typically narrowly to broadly cylindro-clavate, at times clavate to nearly napiform, 24.4–66.1 × 6.8–19.0 µm (x_m = 41.7 × 11.6 µm; E = 2.0–6.9; Q = 3.89; n = 13/1). Stipitipellis a cutis between abundant, densely scattered clusters of colorless 1–3 celled hyphae with clavate, broadly obclavate or broadly cylindro-clavate caulocystidia and scattered clusters of loosely entangled hyphae, 5–10 cells long, with cylindro-clavate to clavate, colorless caulocystidia. Caulocystidia 16.5–66.4 × 7.1–13.9 µm. Stipe tramal hyphae parallel, 101.4–355.5 × 5.4–13.4 µm. Oleiferous hyphae rare in the pileal and stipe trama, absent in the gill trama, reddish-brown in 3% KOH. Pigmentation coarsely externally incrusted on the tramal hyphae of the lamellae, pileus and stipe and in the basal cells of the pileipellis; obscurely internally parietal in the hyphae of the stipitipellis and in the outermost 1–3 cells of the hyphae of the pileipellis.

Ecology and distribution: Scattered to gregarious in hard packed soil in complex mesophyll vine forest in areas with high rainfall; Lake Euramoo, Danbulla National Park, Ceylon and Papua New Guinea.

Collections examined — **AUSTRALIA. Queensland**, Cook Region, Danbulla National Park, Lake Euramoo Track, 17°09′43.0″S 145°37′44.7″E, 763.2 m, 18 March 2009, DL Largent 9662 (mtSSU HQ876551, LSU HQ876529, RPB2 HQ876507); 19 March 2009, DL Largent 9670 (mtSSU HQ876552, LSU HQ876530, RPB2 HQ876508), DL Largent 9671; 21 March 2010, DL Largent 9807 (mtSSU HQ876555, LSU HQ876533, RPB2 HQ876511), DL Largent 9811 (mtSSU HQ876556, LSU HQ876534, RPB2 HQ876512), DL Largent 9812; 24 March 2010, DL Largent 9818, 9827; Cook Region, Danbulla National Park, Kauri Creek Track, 17°07′49.1″S 145°35′58.2″E, 725.1 m, 21 February 2010, DL Largent 9729 (mtSSU HQ876553, LSU HQ876531, RPB2 HQ876509); 13 March 2010, DL Largent 9778 (mtSSU HQ876554, LSU HQ876532, RPB2 HQ876510).

Comments— *Pouzarella lasia* is diagnosed by a typically conic to campanulate to parabolic pileus that is hispid-squamulose on the disc and imbricate squamulose elsewhere and covered when young with pallid to brownish-orange tipped squamules that become dark brownish-gray with age and exposure; a ≤50 mm long stipe covered by an orange-gray to brownish-orange layer of entangled fibrils overlying longitudinally appressed grayish-brown fibrils and with an obscurely strigose base, nodulose-angular basidiospores averaging 11.5 × 7.2 µm, absence of cheilocystidia, presence of aborted basidia that project beyond the hymenium, internally parietal pigmented pileocystidia and caulocystidia, ± thick-walled strongly encrusted basal pileipellis hyphae, and a nondistinctive taste and odor.

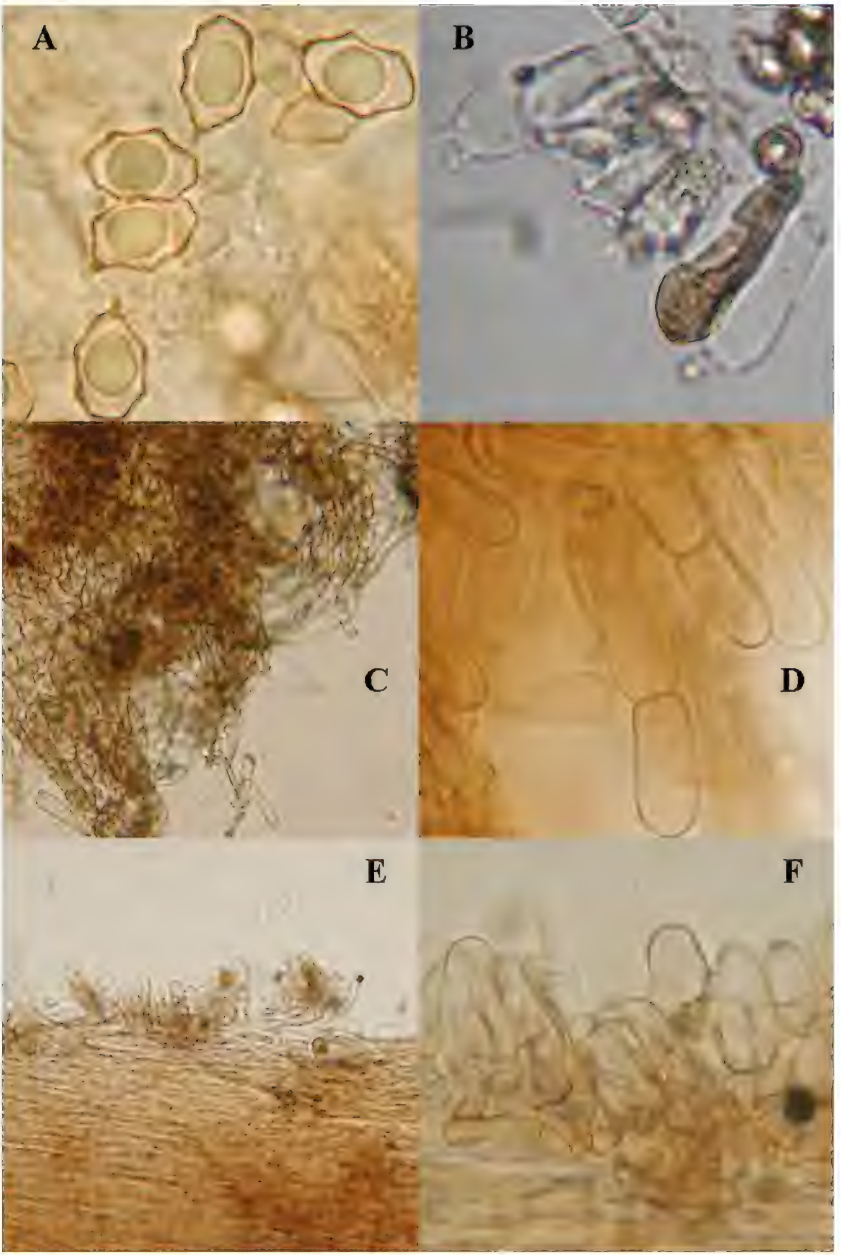

Plate 20 – *Pouzarella lasia* (DLL 9662). A: Basidiospores (1000×); B: Basidia (colorless) and pseudocystidia (dark) (400×); C: Pileipellis on disc (100×); D: Pileocystidia (400×); E: Stipitipellis (100×); F: Caulocystidia (400×).

In Australia, we observed 30–40 *P. lasia* basidiomata in the same habitat and locality twice over a 2–4 day period in mid-March 2009 and mid- to late-March 2010. Most pilei from basidiomata collected during the beginning days possessed light-colored squamules over the entire surface but the squamule base of the remaining basidiomata began to develop dark gray brown tints. Pileal surfaces from basidiomata collected at the end of the 2–4 day period had only dark gray brown squamules and were otherwise morphologically identical to *Pouzaromyces lasius* as described and illustrated by Horak (1980). With the exception of the light colored squamules, the basidioma features from the beginning of the time period also matched those of *Pouzaromyces lasius*. Horak apparently either overlooked the light colored squamules in young specimens or examined only mature specimens.

Horak (1980) and Pegler (1977) studied the holotype of *Agaricus lasius*. Referring to his type study in his *Pouzaromyces lasius* description, Horak noted basidiospores measuring 10.5–13.5 × 6–8 µm, cheilocystidia absent, and the terminal (1–3 cell thick) hairs on stipe [and pileus?] as thin-walled and remaining cells as thick-walled. For the holotype, Pegler (1977) reported basidiospores averaging 14 × 8.5 µm (11.5–15.5 × 8–9.5 µm) and a 4-µm thickness for the cell walls of the pileal hairs. It is rather perplexing to have two investigators study the same specimens and report such distinct differences.

Pouzarella lasia, P. domingensis from the Dominican Republic, *Pouzaromyces napaliensis* from the Hawaiian Islands, and *Entoloma testaceostrigosum* from the state of Kerala in India lack cheilocystidia and have similarly shaped and sized basidiospores. *Pouzarella domingensis* differs in the presence of inflated cells in the pileal subpellis (Baroni et al. 2008), while *Pouzaromyces napaliensis* has aciculate to setiform caulocystidia and pileocystidia, smaller basidiomata (2.5 mm broad pileus, 3–4 × <1 mm stipe), and a pale yellowish-brown pileus (Desjardin & Baroni 1991). *Entoloma testaceostrigosum* is separated by its larger (12.5–17.0 × 7–10 µm) basidiospores and absence of encrusted pileipellis and stipitipellis hyphae (Manimohan et al. 2006).

Pouzarella lasia was found fruiting abundantly in two locales. All these collections were made in a similarly complex mesophyll vine rainforest in Danbulla National Park in northeastern Queensland. Four collections —DL Largent 9662 (18 March 2009), 9670 (19 March 2009), 9807 and 9811 (21 March 2010)— came from populations in identical habitats on the Lake Euramoo Track, while two —DL Largent 9729 (21 February 2010), 9778 (13 March 2010)— were from the same population and identical habitat on the Kauri Track. We found no evidence of genetic isolation among populations separated by 4 km and the level of allelic variation within each locus is consistent with the variation expected in interbreeding populations with a large effective population size. Future studies of these and other populations

of *P. lasia* may provide insight into the extent of gene flow or genetic isolation among populations.

See *P. setiformis* for comparisons with Australian taxa related to *P. lasia*,.

Discussion

In the past decade, efforts to gain insight into the morphological features that unite mushroom-forming fungi with their sequestrate relatives (*Basidiomycota, Agaricales*) have pinpointed some unique characters that can form the basis of natural classifications (e.g. Moncalvo et al. 2004, Matheny et al. 2006). However, defining these boundaries for large and diverse families such the *Entolomataceae* with over 1500 mushroom-forming species and few sequestrate taxa (Largent 1994, Moncalvo et al. 2004, Co-David et al. 2009) can be challenging. The basis for the early classification of all members in the *Entolomataceae* is the pinkish basidiospore deposit and angular basidiospores observed in polar view, features that may form the basis of a natural classification based on the observed monophyletic support using DNA sequence data (Moncalvo et al. 2004, Matheny et al. 2006, Co-David et al. 2009).

Despite the support for the monophyly of the *Entolomataceae*, defining generic boundaries within the family may be challenging. For example, several of the proposed generic concepts based on morphological features have been questioned based on the lack of observed monophyletic support in phylogenetic analyses (Moncalvo et al. 2002, 2004, Co-David et al. 2009). Large genera that exemplify these problems include *Entoloma* (Fr.) P. Kumm., recovered as either polyphyletic (Moncalvo et al. 2004) or paraphyletic (Co-David et al. 2009). A similar lack of support has been observed for *Claudopus* Gillet, *Leptonia* (Fr.) P. Kumm., *Rhodocybe* Maire, and *Nolanea* (Fr.) P. Kumm. (Moncalvo et al. 2002, Co-David et al. 2009). Although it is unquestionably an under-sampled genus, monophyly of *Pouzarella* is supported by molecular data (from only two collections, the European *Entoloma araneosum* and Indian *E. violaceovillosum*; Co-David et al. 2009). In this paper, we include a phylogenetic analysis of two European (Co-David et al. 2009) and ten Australian *Pouzarella* species; given the exclusion of other entolomataceous genera, we understand that our phylogeny neither refutes nor supports the monophyly of *Pouzarella.* Nonetheless, our sequence analyses support delineation of the ten Australian species based on micromorphological features, and features common to these clades are highlighted as characters that may form the basis of a natural classification for other genera.

One interesting pattern apparent in the phylogeny is the strong support for the monophyletic clade containing *P. lageniformis*, *P. parvula,* and *P. pilocystidiata*. All species possess long cylindro-clavate pileocystidia, abundant thin-walled lageniform to ventricose-rostrate cheilocystidia, and lack aborted

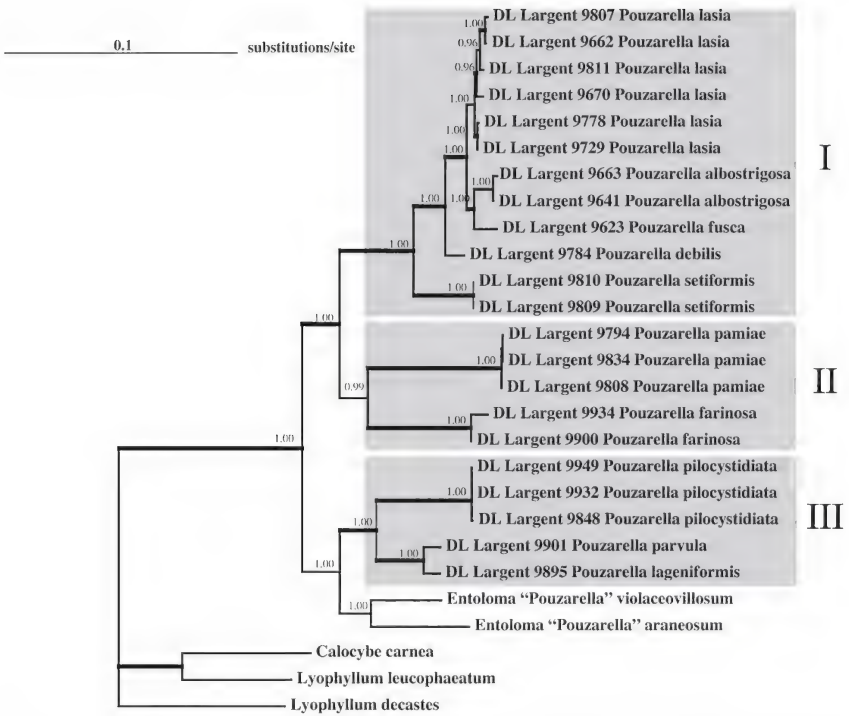
0.1
substitutions/site
DL Largent 9807 Pouzarella lasia
DL Largent 9662 Pouzarella lasia
DL Largent 9811 Pouzarella lasia
DL Largent 9670 Pouzarella lasia
DL Largent 9778 Pouzarella lasia
DL Largent 9729 Pouzarella lasia
DL Largent 9663 Pouzarella albostrigosa
DL Largent 9641 Pouzarella albostrigosa
DL Largent 9623 Pouzarella fusca
DL Largent 9784 Pouzarella debilis
DL Largent 9810 Pouzarella setiformis
DL Largent 9809 Pouzarella setiformis
I
DL Largent 9794 Pouzarella pamiae
DL Largent 9834 Pouzarella pamiae
DL Largent 9808 Pouzarella pamiae
DL Largent 9934 Pouzarella farinosa
DL Largent 9900 Pouzarella farinosa
II
DL Largent 9949 Pouzarella pilocystidiata
DL Largent 9932 Pouzarella pilocystidiata
DL Largent 9848 Pouzarella pilocystidiata
DL Largent 9901 Pouzarella parvula
DL Largent 9895 Pouzarella lageniformis
III
Entoloma "Pouzarella" violaceovillosum
Entoloma "Pouzarella" araneosum
Calocybe carnea
Lyophyllum leucophaeatum
Lyophyllum decastes

Plate 21 – Phylogenetic relationships of *Pouzarella* based on Maximum Likelihood analysis of mtSSU, LSU, and RPB2 of 10 Australian, one European, and one Indian species. Tree topologies based on Maximum Likelihood and Bayesian estimation were congruent. Thick branches represent nodes that are supported by bootstrap values of 70% or greater and values above each branch are the Bayesian posterior probability values computed from the 50% majority rule consensus tree. Shaded areas represent the three clades recovered. Taxa are labeled with the collection identification number and name of taxon.

basidia (unusual in *Pouzarella*). Strong support was also observed for another clade comprising *P. albostrigosa*, *P. debilis*, *P. fusca, P. lasia,* and *P. setiformis*. One unique morphological feature common to this clade is the conspicuous absence of cheilocystidia, a feature shared by other tropical species including *Pouzaromyces minutus* (Horak 1980), *Pouzaromyces napaliensis* (Desjardin & Baroni 1991), and *Entoloma testaceostrigosum* (Manimohan et al. 2006). *Pouzarella pamiae* and *P. farinosa* formed a monophyletic group, although with low ML BS support. Both possess a squamulose-hispid stipe surface and ± thick walled cheilocystidia — features also encountered in the tropical *P. ferreri* and temperate *P. dysthales* (Peck) Mazzer (Baroni et al. 2008, Noordeloos 2004). Future studies that include these and other representative *Entolomataceae* taxa are needed to better assess phylogenetic relationships and diagnostic

morphological characters that could form the basis of a natural classification of *Pouzarella*.

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Key to *Pouzarella* species from central New South Wales (N) and northeastern Queensland (Q)

1. Cheilocystidia present; aborted basidia absent or if present, rare and colorless in 3% KOH ..... 2 (Clade II, III)
1. Cheilocystidia absent; aborted basidia present and typically abundant and staining dark reddish-brown in 3% KOH ..... 6 (Clade I)
2. Cheilocystidia acicular, aculeate, narrowly acuminate, finally rostrate ventricose; pileocystidia cylindro-clavate, with Q = 7.0 or more ..... 3 (Clade III)
2. Cheilocystidia globose, napiform, vesiculose pyriform or clavate to broadly acuminate; pileocystidia versiform, with a Q <5.0 ..... 5 (Clade II)
3. Pileus orange white to grayish-orange; stipe apex pruinose; taste mild;..... 4
3. Pileus squamules and fibrils dark brown on dark blond to brown background; stipe apex tomentulose to densely hirsute or with abundant bundles of fibrils; taste farinaceous ..... 1. *P. pilocystidiata* (N)
4. Pileus hispid-squamulose; stipe entirely pruinose; pileocystidia at most acuminate ..... 2. *P. lageniformis* (N)
4. Pileus entirely densely matted squamulose; stipe pruinose at the apex, glabrous elsewhere; at least some pileocystidia setiform ..... 3. *P. parvula* (N)
5. Pileus at first entirely reddish-golden to brownish-orange, then darkening to medium teak brown to burnt umber; cheilocystidia broadly clavate to broadly acuminate ..... 4. *P. pamiae* (Q)
5. Pileus with densely packed upright tufts of orange gray squamules on a grayish-brown background, overall light grayish-brown; cheilocystidia at first nearly globose then napiform, vesiculose, or pyriform.. 5. *P. farinosa* (N)
6. Stipitipellis with scattered to abundant setiform caulocystidia; pileipellis with setiform pileocystidia, most evident at the margin ..... 6. *P. setiformis* (Q)
6. Stipitipellis with cylindric to clavate to obclavate caulocystidia; setiform caulocystidia and pileocystidia absent 7
7. Pileus broadly convex to convex; stipe up to 22 mm long; aborted basidia embedded in the hymenium or rarely extending beyond the hymenium by 6–7 µm 8
7. Pileus conic, campanulate or parabolic; stipe ≤50 mm long; aborted basidia extending beyond the hymenium by ≤17 µm ..... 10. *P. lasia* (Q)
8. Caulocystidia of one type, similar to the pileocystidia ..... 7. *P. debilis* (Q)
8. Caulocystidia of two types: 1) cylindro-clavate, the end cells of loosely entangled hyphae, similar to the pileocystidia; 2) clavate, obclavate, or broadly napiform and typically solitary and not in loose hyphal clusters 9
9. Pileus minutely but entirely squamulose, black to a very dark gray-brown, without off-white squamules when young; pileus opaque when mature; stipe base pallid and obscurely strigose ..... 8. *P. fusca* (Q)
9. Pileus entirely hispid-squamulose and with off-white (5A-B1-2) squamules when young, base color dark grayish-brown; pileus striate when mature; stipe base white and distinctly strigose ..... 9. *P. albostrigosa* (Q)

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Amanita vernicoccora sp. nov. —the vernal fruiting 'coccora' from California

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Abstract — We describe *Amanita vernicoccora,* a vernal fruiting species known as the "spring coccora" in California. Sequence analyses of four DNA regions and phenotypic traits demonstrate that *A. vernicoccora,* long considered a pale-colored form of the autumnal fruiting *A. calyptroderma* (the 'fall coccora'), is a unique species. Morphological and genetic data support both species in section *Caesareae.* We also address some nomenclatural and taxonomic intricacies surrounding application of the proper name for the fall-fruiting coccora.

Key Words — *Amanitaceae,* fungal taxonomy, nrLSU, nrITS data

Introduction

The popular name —'coccora' or 'coccoli'— was coined by Italian-Americans in California for two valuable edible mushrooms of genus *Amanita* Pers. collected during the fall and spring. The autumnal fruiting *A. calyptroderma* (referenced here as the 'fall coccora,' Fig. 1a) and the vernal fruiting *A. vernicoccora* (referenced here as the 'spring coccora,' Fig. 1b) are closely related and were for many years considered conspecific seasonal color forms. Both taxa represent subg. *Amanita* sect. *Caesareae* Singer and are close relatives of *A. caesarea* (Scop.) Pers. —the European 'Caesar's amanita'— a popular edible in Europe, especially in Italy. The coccora has a notable thick cottony veil that leaves a distinctive monolithic velar remnant on the pileus. The association with a thick-walled cocoon enveloping the *Amanita* egg is the etymological origin of the popular name.

Due to the perceived conspecificity of the two coccora, the autumnal name was used for both species, currently referenced as either *A. calyptroderma* or *A. lanei.* Both names are still used in various reference materials and field

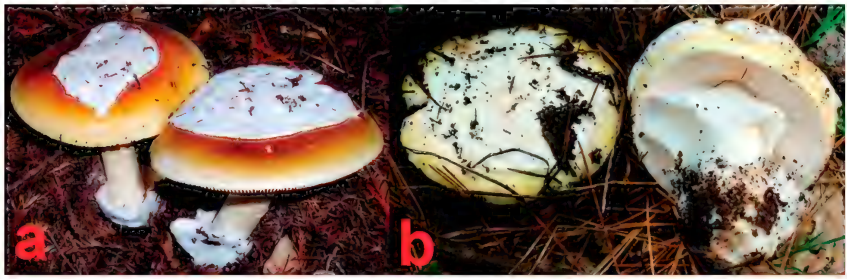

Fig. 1. a) The fall coccora *Amanita calyptroderma*. b) The spring coccora *Amanita vernicoccora*.

guides, and there is general confusion as to which is preferable. The key details of the rich and intricate nomenclatural and taxonomic history surrounding the choice of the proper scientific name for the fall coccora are discussed here.

Nomenclatural and taxonomic history of the fall coccora

Amanita calyptroderma G.F. Atk. & V.G. Ballen, Science, n.s. 29: 944. 1909.
?= *Amanita calyptrata* Peck, Bull. Torrey Bot. Club 27: 14. 1900 [nom. illegit., non Lam. 1783].
≡ *Venenarius calyptratus* Murrill, Mycologia 4: 241. 1912 [nom. nov.].
≡ *Venenarius lanei* Murrill, N. Amer. Fl. 10: 75. 1914 [nom. nov. superfl.].
≡ *Amanita lanei* Sacc. & Trotter, Syll. Fung. 23: 5. 1925 [nom. nov.].

The fall coccora was first validly named as *Amanita calyptrata* (Peck 1900) based on autumnal collections from fir woods in Oregon and notes by Dr. H. Lane. When first published, this name was legitimate, but under the retrospective application of the current International Code of Botanical Nomenclature (ICBN Art. 53.1; McNeill et al. 2006), it is an illegitimate later homonym of *A. calyptrata* Lam. Murrill (1912) "recombined" the name as *Venenarius calyptratus* —under ICBN Art. 58.1, this must be interpreted as a legitimate nom. nov., with priority dating from 1912. Two years later, Murrill (1914) had become aware of the later homonymy of Peck's binomial (illegitimate under the 1907 American Code of Botanical Nomenclature) and published *V. lanei* as a nom. nov. based on Peck's description and typification. However, under ICBN Art. 52.1, the name *V. lanei* was a superfluous (i.e., illegitimate) synonym of *V. calyptratus*, and consequently the "recombination" *Amanita lanei* published by Saccardo & Trotter (Trotter 1925) must also be interpreted as a legitimate nom. nov. under ICBN Art. 58.1.

In 1909 George Atkinson described *A. calyptroderma* (Atkinson 1909a,b) based on autumnal collections, several diagnostic photographs of fresh material, and supplemental notes from Virginia Ballen from Brookdale, Santa Cruz Co., California. According to the Atkinson papers in the CUP Herbarium, the collections were sent from California in fresh condition and

arrived at Cornell University on Dec 7, 1908 with some basidiomata partly decayed. The major elements of the collection notes match the *A. calyptroderma* protologue (Atkinson 1909a,b). The date of the collections, the description, and the photographs published in Atkinson (1909c) leave no doubt that the name *A. calyptroderma* was applied to the familiar fall coccora (Fig.1a, Fig. 10).

Apparently, Atkinson (1909c) was aware of Peck's earlier species and makes a single reference to *A. calyptrata* in the footnote of his description —"closely related to *Amanita calyptrata* Peck, Bull. Torr. Bot. Club 27:14.1909 [sic, = 1900], but differs in color and other characters." Contrary to Atkinson's view, most resident California taxonomists consider *A. calyptroderma* and *A. lanei* (≡ *A. calyptrata*) conspecific. Taxonomically, we believe that *A. calyptroderma* faithfully represents the fall coccora. Moreover, if we accept conspecificity with *A. lanei*/*V. calyptratus* (dating from 1912), *A. calyptroderma* (dating from 1909) has nomenclatural priority. [It should be noted that under the ICBN, until 1981 the correct name for this conspecific taxon was *Amanita calyptrata*, because it remained legitimate and had priority over *A. calyptroderma*. This situation was reversed when the 1981 International Botanical Congress (Sydney) moved the starting point date for agaric nomenclature back from 1821 to 1753, making *A. calyptrata* Peck a nom. illegit.]

Nevertheless, there are lingering doubts about the exact identity of *A. lanei*, as the original description by Peck notes green tinges on the pileus. In our experience, the fall coccora frequently develops olive-brown tints in age or when exposed to colder weather but we would not describe the color as green (Fig. 10c). Olive-brown tints, for example, are commonly observed on specimens from the Sierra Nevada foothills. It is also possible that the stronger color qualification of "green" was used instead of "olive," a common style in many early descriptions. Nonetheless, the green color has been stressed in numerous taxonomic treatments as an important diagnostic character. Zeller (1931) states: "These two species, *A. calyptrata* Peck and *A. calyptroderma* Atkinson and Ballen, are very similar and difficult to distinguish. The main distinction is the greenish tinting of the pileus and gills in *A. calyptrata*..." Hotson (1936) supported this view that the green tints were a key diagnostic character in his treatment of the amanitas of Washington. Although it is unclear whether these authors personally collected green- tinted specimens, we cannot discount the possibility that *A. lanei* refers to a rarely collected unique species from the more northern areas of the Pacific Northwest. This prospect is additionally corroborated by the suggested association of *A. lanei* with fir per its original description. In contrast, the fall coccora is primarily associated with broadleaved trees in California. Therefore, we believe that on both nomenclatural and taxonomic grounds, the correct name for the fall coccora is *A. calyptroderma*.

Murrill (1912, 1914) and Zeller (1931) synonymized *Amanita calyptroderma* and *A. calyptratoides* Peck, although it appears that Murrill never collected or examined either species. Current researchers do not accept this synonymy, and we also consider it to be clearly an error. The macromorphological traits of *A. calyptratoides* —such as the relatively smaller size (4–8 cm), gray-brown to plumbeous pileal colors, and particularly the evanescent to inconspicuous annulus— contrast sharply with the features of *A. calyptroderma* as supported by the very diagnostic original photographs (Atkinson 1909c). The current interpretation of *A. calyptratoides* refers to a small species, common under oaks, with primary distribution of southern California and Mexico (Tulloss 2011).

The conspecificity of fall vs. spring coccora

The conspecificity of the fall and spring fruiting coccora was assumed at the time of the first descriptions and that view generally has not changed. While Peck and Murrill do not seem to have been aware of the existence of a spring fruiting species, Atkinson was and discussed it at length. The original description of *A. calyptroderma* (Atkinson 1909a,b) clearly referenced the fall species based on the time of collection and the colors he specified — "pileus maize yellow to pale chrome yellow." Very soon after that, based on the input of his experienced California correspondent, Virginia Ballen, he offered a more elaborate description of the pileus colors (Atkinson 1909c), including the spring form, "The pileus is a maize-yellow in its bright-colored forms and varies to a pale straw color or Naples yellow (R.) in the vernal forms."

Regarding the vernal fruiting habit of the coccora, Atkinson (1909c) developed an elaborate hypothesis:

> "It occurs in the high Sierras and in the Coast Range. Probably the entire summer season is needed for the growth and extension of the mycelium in the forest mold, so that the huge fruit bodies are developed in late autumn and early spring. While we have as yet no information bearing on the time of origin of the fundament of the fruit bodies, it is likely that all of them are formed during the summer and late autumn, and that the second crop, which appears early in the spring, is composed of plants which have lived through the winter in a partially developed condition. The autumn crop ceases about the last of December, while the spring crop begins about the middle of March."

Contemporary authors of influential field guides in California (Arora 1986, Wood & Stevens 2009) continue to reflect the notion of the conspecificity and seasonal color differences between the fall and spring coccora. At the same time, the awareness that they are likely different species has grown in recent years. Smith et al. (2007) first reported that the fall and spring coccora differ significantly in their ITS sequences.

Materials & methods

Methods for morphological studies and DNA extraction, PCR conditions and primers, and amplicon clean up and sequencing follow Bojantchev & Davis (2011). Collections are stored in the Bojantchev private herbarium or at the University of California herbarium in Berkeley (UC) as noted.

All *Amanita* nrITS and nrLSU sequences from the public databases GenBank (http://www.ncbi.nlm.nih.gov) and UNITE (http://unite.ut.ee/) were downloaded, reviewed, and selected for quality and identification congruity. Sequence alignments were generated with MAFFT v6.821b (Katoh et al. 2002) with the G–INS–i global alignment iterative refinement strategy. No gap opening and extension penalties were set for better resolution of the variable sectors within nrITS, while the default higher penalties were left for the more conserved nrLSU region. The alignments were visually inspected and corrected where needed.

The evolutionary history of 248 *Amanita* nrLSU sequences was inferred using the Maximum Likelihood method based on the Tamura-Nei model as implemented by MEGA5 (Tamura et al. 2007).

Nine *Amanita* sect. *Caesareae* nrITS sequences, representing four taxa, were selected for higher resolution phylogenetic analysis (Fig. 3). *Amanita muscaria* (L.) Lam. was selected as outgroup. Six sequences representing four taxa —the Asian *Amanita hemibapha* (Berk. & Broome) Sacc., *A. caesarea, A. calyptroderma, A. vernicoccora*— were sourced from GenBank. Three sequences of *A. calyptroderma* and *A. vernicoccora* (including the holotype) are from the authors' collections. Two nrITS sequences of *Amanita jacksonii* Pomerl. (GenBank: AY436461, HQ539807) were excluded due to quality and coverage concerns. The evolutionary history was inferred using the Maximum Parsimony method as implemented by MEGA5 (Tamura et al. 2007). The MP trees were generated by the Close-Neighbor-Interchange algorithm with search level 0 in which the initial trees were obtained with the random addition of sequences (10 replicates). The percentage of replicate trees in which the associated taxa clustered together was calculated from a bootstrap test with 1000 replicates. The search resulted in nine most parsimonious trees (length = 187), which differed only in the topology of the terminal nodes.

Phylogenetic results

Four gene regions —nrITS, nrLSU, RPB2, β-tubulin— were sequenced from *A. calyptroderma* (RMD07036, not photographed) and *A. vernicoccora* (RMD07020, Fig. 9b). Direct comparisons of these four gene regions support

Table 1. The four DNA regions sequenced from collections of *Amanita calyptroderma* and *A. vernicoccora*.

DNA regions	*A. calyptroderma* [coll. RMD07036]	*A. vernicoccora* [coll. RMD07020]	BLAST maximum identity index difference
nrITS	GQ250400	GQ250401	4%
nrLSU	GQ250415	GQ250416	2%
RPB2	GQ401358	GQ401359	2%
β-tubulin	GQ401356	GQ401357	4%

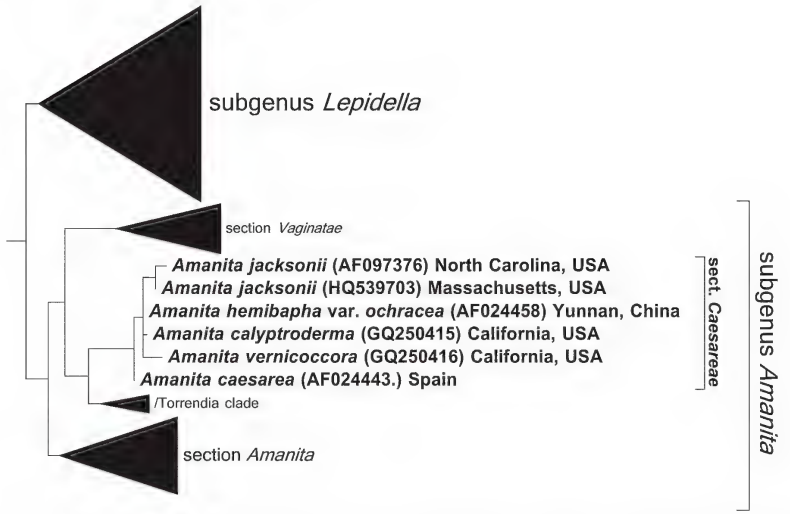

Fig. 2. Phylogenetic tree inferred by maximum likelihood analysis of 248 *Amanita* nrLSU sequences. The tree shows the position of sect. *Caesareae* relative to the collapsed subtrees of the other *Amanita* subgenera and sections. GenBank accession numbers are enclosed in brackets.

the phenotypic and ecological determination that taxonomically they are different species and have been on separate evolutionary paths (Table 1).

The Maximum Likelihood tree (Fig. 2) shows the position of sect. *Caesareae* within genus *Amanita* with the major subgenera and sections compressed. Our research shows strong phylogenetic support for sect. *Caesareae* and generally agrees with Drehmel et al. (1999) and Justo et al. (2010). One most parsimonious MP tree is given in Fig. 3.

Fig. 3. Phylogenetic tree inferred by maximum parsimony analysis of nine *Amanita* sect. *Caesareae* nrITS sequences with *A. muscaria* as the outgroup. The tree shows the position of *A. vernicoccora* relative to its closest neighbors in sect. *Caesareae*. The percentages of clustered replicate trees based on the bootstrap test (1000 replicates) are shown above the branches while the branch lengths representing estimated nucleotide substitutions are shown below. GenBank accession numbers are enclosed in brackets. The type collection is marked with *.

Fig. 4. *Amanita vernicoccora* DBB00201.

Taxonomy

Amanita vernicoccora Bojantchev & R.M. Davis, **sp. nov.** Figs 4–8

MycoBank MB 561705

Pileo 60–180 mm lato, hemispherico, dein planoconvexo, glutinoso, flavo, margine striato, cum fragmento volvae magno persistento albo. Lamellis confertis, liberis, albis. Stipite 50–140 mm longo, cylindrico, albo. Annulo superiore, striato, albo. Volva ampla, membranosa, crassa. Carne albido. Sapore miti. Species vernalis. Sporis 9–12 × 6–7 µm, late ellipsoideis, hyalinis. Basidiis 42–64 × 11–14 µm, tetrasporigeris, fibulis praesentibus.

Type: USA. California: El Dorado County, Georgetown, Dru Barner Campground, under *Quercus kelloggii,* 21 May 2011, Bojantchev DBB43538 (**Holotype** UC 1860906; Genbank nrITS JN133297).

Etymology: from the Latin *vernus* = vernal and *coccora* = the local name for this mushroom.

Pileus 60–180 mm diam., hemispherical to convex when young, plano-convex to plano-concave with age; margin straight, short striate; color uniform yellow to pale yellow; with a white, monolithic, central velar remnant, cottony thick at first, thinning with age. Lamellae crowded, 10–18 mm broad, white to pale cream, even, seceding to free, lamellulae common. Stipe 50–140 mm long, 15–30 mm wide, cylindrical, white with yellow tints at age, context hollow or stuffed with a cottony or jelly-like substance in buttons. Annulus superior, membranous, pendant, upper surface striate, cottony-thick at first, thinning and collapsing with age, white to pale yellow with age. Volva ample, thick, friable, free, sac-like at first, thinning and collapsing with age, white. Context white to pale yellow. Odor mild at first, pungent with age, frequently interpreted as fishy. Taste mild to pungent. Macrochemical Reactions 5% KOH negative,

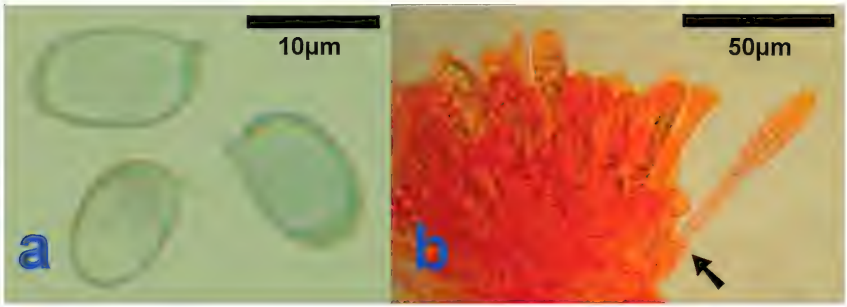

Fig. 5. *Amanita vernicoccora* (UC 1860906, holotype): a) Basidiospores. b) Hymenial layer composed of basidia, basidioles, and subhymenial layer formed of densely packed irregular to pyriform cells; clamped basidia (see arrow). Tissues are stained in Congo Red.

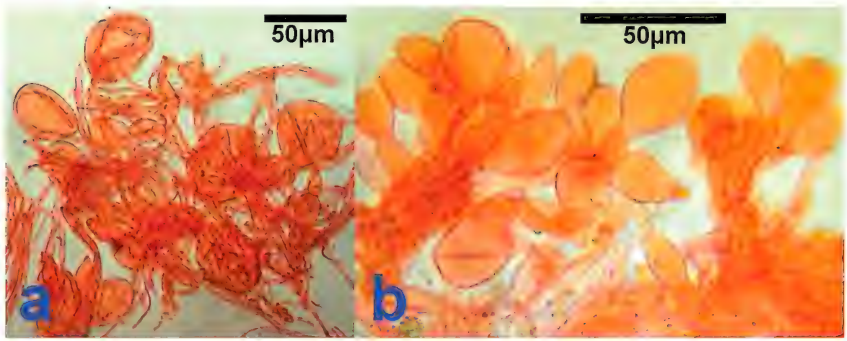

Fig. 6. *Amanita vernicoccora* (UC 1860906, holotype): a) Filamentous and vesiculose cells from the outer surface of the universal veil. b) Inflated cells from the upper surface of the annulus.

10% NH_4OH negative, 3% phenol slowly pinkish-lilac on all surfaces, Guaiac negative, 10% $FeSO_4$ negative. Spore Deposit white.

Basidiospores (8.5–)9.2–11.8(–12.5) × (5.7–)6.2–7.1(–7.5) µm (mean 10.5 × 6.5 µm), Q = 1.43–1.77, Q_{av} = 1.61 (N = 183, 5 basidiomata, 4 collections), broadly ellipsoid, with a prominent lateral apiculus, hyaline, inamyloid. Basidia 42–64 × 11–14 µm, 4-spored, clavate, clamped, sterigmata 4–6 µm long. Subhymenial layer composed of several layers of irregular to pyriform cells 10–30 × 8–22 µm, frequently clamped Lamellar trama divergent, composed of filamentous to swollen hyphae 8–24 µm wide, occasionally clamped. Cystidia not observed. Pileipellis an ixocutis to ixotrichoderm, densely interwoven within a gelatinous matrix, 200–320 µm thick, hyphae 2–7 µm wide, branched, clamped, intracellular pigment. Annulus composed of filamentous cells, 2–6 µm wide with clusters of inflated clavate, pyriform or mucronate cells 25–50 × 16–24 µm, mainly on the upper surface. Universal Veil formed of dense filamentous hyphae 1.5–8 µm wide, clamped, interspersed with vesiculose cells

— on the inner surface: narrow ellipsoid to elongated, 50–120 × 20–50 µm; on the outer surface: broadly ellipsoid to subglobose, 60–180 × 40–150 µm, slightly gelatinized. Stipe Trama acrophysalidic, composed of filamentous hyphae 2–8 µm wide and inflated hyphae 50–150 × 20–46 µm, occasionally clamped.

Habitat and distribution — *Amanita vernicoccora* fruits in the late winter and spring and is apparently restricted to California. Along the coast and in the lower elevations of Sierra Nevada (<2000 feet) it fruits in February–March and is known to associate with evergreen oaks such as live oak (*Quercus agrifolia* Née), interior live oak (*Quercus wislizenii* A. DC.) and blue oak (*Quercus douglasii* Hook. & Arn.). Likely associations are also to Pacific madrone (*Arbutus menziesii* Pursh) and manzanita (*Arctostaphylos manzanita* Parry). Infrequently, *A. vernicoccora* fruits in the northern coastal areas where it is likely associated with tanoak (*Notholithocarpus densiflorus* (Hook. & Arn.) Manos et al. This species is far more common in the foothills of the Sierra Nevada and Shasta Cascade range in May–June where the primary association in the lower elevations (2000–4000 feet) is with the deciduous black oak (*Quercus kelloggii* Newb.). At higher elevations *A. vernicoccora* may switch association to conifers, but we have not seen it outside of the range of the black oak (≤6000 feet).

Additional collections examined: **USA. California**: Amador County, Indian Grinding Rock State Park, elev. 2400 ft, under black oak, 17 May 2009, Davis RMD07020 (Genbank: nrITS GQ250401, nrLSU GQ250416, RPB1 GQ401359, β-tubulin gene GQ401357); El Dorado County, Georgetown, Dru Barner campground, elev. 2540 ft, under black oak, 26 May 2011, Davis RMD110003; 29 May 2011, Bojantchev DBB43625; Eldorado National Forest, Crystal basin area, near Union Valley Reservoir, elev. 4870 ft., under mixed conifers and black oak, 2 Jun 2006, Bojantchev DBB00118; Calaveras County, Stanislaus National Forest, off Hwy. 120 and Hardin Flat Road exit, elev. 3600 ft., under mixed conifers and black oak, 27 May 2006, Bojantchev DBB00102; Stanislaus National Forest, off Evergreen Rd, elev. 4100 ft. under mixed conifers and black oak, 22 May 2006, Bojantchev DBB00099; Siskiyou County, Dunsmuir, off Hwy 5, near Dunsmuir airport, elev. 2300 ft. under black oak, 31 May 2008, Bojantchev DBB00201; Marin County, Marin Watershed, off Bolinas-Fairfax Rd., elev. 800 ft. under live oak, 13 Mar 2008, Bojantchev DBB07809.

Fig. 7. *Amanita vernicoccora:*
a) UC 1860906, holotype. b) DBB07809 under live oak in the California coastal range.

FIG. 8. *Amanita vernicoccora* — a study in the development of the basidiocarp: a) Expanding button, note the jelly like substance in the stipe context, which later turns cottony or leaves it hollow. b) Note the thick cottony universal veil and the striate upper surface of the annulus. c) Note the thinning of the universal veil on pileus, friable volva and collapsing annulus.

FIG. 9. *Amanita vernicoccora* collections: a) RMD110003. b) RMD07020.

FIG. 10. *Amanita calyptroderma*, the fall coccora: a) DBB00347. b) DBB00393. c) DBB00491 shows the frequent olive tinges on the pileus due to exposure to cold weather.

Discussion

Both *A. vernicoccora* and *A. calyptroderma* are beautiful species, distinguished by a thick, cottony, white velar remnant on the pileus that remains whole into maturity. This feature makes them among the easier amanitas to identify. The edibility of the fall coccora, *A. calyptroderma*, was extolled by both Peck (1900) and Atkinson (1909a), who made references to its gastronomical virtue.

While considered conspecific, *A. vernicoccora* also was presumed edible and is widely collected for the table. Even though practical experience has shown that both coccora are relatively safe choices as edibles, confusion with other toxic *Amanita* species do occur and the possibility of dangerous misidentification must be stressed. The standard word of caution is that several reliable taxonomic factors must be evaluated before an *Amanita* collection is deemed safe for consumption.

The greatest danger posed by the pale spring coccora is misidentification with members of the deadly toxic sect. *Phalloideae* (Fr.) Quél. —*Amanita ocreata* Peck and *A. phalloides* (Fr.) Link, which also associate with oaks throughout California. *Amanita ocreata* is a native species, while *A. phalloides* is an import from Europe that has found favorable conditions and spread widely in California (Pringle et al. 2009). Both can have a large patch of universal veil on the cap (Fig. 11). In southern California, *A. ocreata* develops a much more

Fig. 11. Dangerous look-alikes of *A. vernicoccora:* a) The deadly toxic *Amanita ocreata* fruits in the spring under oaks. b) The deadly toxic *Amanita phalloides* is a native of Eurasia, but a recent import to California that fruits abundantly under oak in late fall and winter.

Fig. 12. Dangerous look-alikes of *A. vernicoccora:* a) The toxic *Amanita gemmata* gr. b) The toxic *Amanita aprica* is frequently found with *A. vernicoccora* in the Sierra Nevada foothills. The minutely broken velar remnants on the pileus contrast with the monolithic veil remnant of the spring coccora.

yellowish pileal coloration, which may further confound field identification. Attention needs to be paid to all features of the basidiocarp, particularly to the almost complete absence of striations on the pileal margin of the *Phalloideae* species.

Other yellow species that can be mistaken for *A. vernicoccora* are in the *Amanita gemmata* (Fr.) Bertill. clade, or gemmatoid amanitas (Fig. 12a), which fruit in both the fall and spring in California. They also are toxic. In the Sierra foothills, *A. vernicoccora* frequently co-occurs with *Amanita aprica* J.E. Lindgr. & Tulloss (Fig. 12b). One key feature separating the two species is the manner in which the veil of gemmatoid amanitas breaks into many small patches and warts.

An extensive iconography of *A. vernicoccora* is available at http://www.mushroomhobby.com.

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Book reviews and notices

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Introduction

The emphasis of this instalment is on tropical mycology, with mycofloristic accounts of Panama and a National park in Malaysia, a revision of the *Boletales* species described by Corner, and cercosporoid taxa from India. The European *Strophariaceae* are the focus of a new book in the series Fungi Europaei, and the economically important *Aspergillus* is the topic of a new volume of Studies in Mycology.

This contribution concludes with one new book announcement.

Ascomycetes

Taxonomic studies in the genus *Aspergillus*. Edited by R.A. Samson, J. Varga & J.C. Frisvad. 2011. Studies in Mycology no. 69. CBS-KNAW Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands. <info@cbs.knaw.nl>. Pp. iv + 97, illustr. ISBN 978-90-70351-86-1. Price: 40 €.

There have been so many publications on *Aspergillus* systematics in recent times, not least "*Aspergillus* Systematics in the Genomic Era" (Studies in Mycology no. 59, 2007; see Mycotaxon 107: 509–511, 2007), that it might have been assumed that most species had now been discovered and named. This collection of six papers, all involving the world's most respected authorities on the genus, shows that is far from the case: in even such an exceptionally well

* Books for consideration for coverage in this column should be mailed to the Book Review Editor at the address above. All unsigned entries are by the Book Review Editor.

studied genus, we have still a long way to go to approach a complete global inventory.

In sect. *Nigri,* four new species are described, taking the total to 26; additionally *A. foetidus* is placed as a synonym of *A. niger,* and molecular data reveal that two further named species are colour mutants of others. Within the black species, a separate study shows that growth rate parameters and hydrolase activities are characters that can assist in species separations in the group. In addition, an L-arabinose transcriptional activator regulating the pentose catabolic pathway and involved in enzyme release is described for *A. niger;* it appears to be confined to *Eurotiales* and to have arisen by gene duplication when the order (or family) split from other filamentous ascomycetes. In sect. *Terrei,* several taxa previously treated as subspecies or varieties of *A. terreus* prove to be distinct lineages and are recognized as separate species, necessitating the introduction of three new names; an additional species is newly described, and the teleomorph-producing *Fennellia nivea* is found to belong elsewhere. Sect. *Flavi* is considered to include 22 species, amongst which are several formerly placed in other sections, and two new aflatoxin-producers distinguished by extrolite profiles as well as sequence data; these have cholesterol-reducing and hepatoxic or neurotoxic extrolites. Finally, 21 species are accepted in sect. *Usti,* of which five are described as new. That section includes two species with teleomorphs —*Emericella heterothallica* and *Fennellia monodii*— for which binominals in *Aspergillus* (with a new combination for *A. monodii*) are used in anticipation of the changes in the CODE (now the INTERNATIONAL CODE OF NOMENCLATURE FOR ALGAE, FUNGI, AND PLANTS) subsequently effected in July 2011.

The papers also demonstrate the value of the polyphasic approach, using several different gene sequences, extrolite, cultural, and other features in species circumscriptions. It was also gratifying to see such an almost seamless transition to one name for one fungus adopted here by the key workers on these organisms, which augurs well for other cases where a monophyletic group has a particularly familiar anamorph-typified generic name. As has become the norm in the STUDIES, all papers are illustrated by exceptionally fine photographs of conidial heads and colonies, many in colour, and an occasional SEM micrograph. All struggling to identify *Aspergillus* species in the sections treated will need to consult a copy, either by having one to hand or accessing it free of charge through the CBS website.

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Cercosporoid fungi of India. By Kamal, 2010. Bishen Singh Mahendra Pal Singh, 23A New Connaught Place, Dehre Dhun, 248 001 Uttarakhand, India. Pp. vii + 351, figs 47, pl. 1. ISBN 978-81-211-0753-2. Price: US$ 90.

Kamal, a former professor at Gorakhpur University, has been involved in the documentation of Indian leaf-inhabiting fungi for over 30 years. In such speciose groups as the cercosporoid fungi, in which over 3000 species have been described, periodic regional syntheses are necessary to translate global monographic work into those accessible at other geographical scales. Following the landmark monograph of Chupp (1954), which accepted 1419 species, an account of the Indian representatives was prepared by Vasudeva (1963), who enumerated 260 species. Now, following on from the critical compilation of Crous & Braun (2003; see Mycotaxon 89: 518, 2003), Kamal has similarly provided an update for India. Of the 1815 cercosporoid fungi reported to have been recorded from India up to 2009, 1050 are accepted, including descriptions of four new species. These are dispersed between the genera *Asperisporium* (5 spp.), *Cercospora* (254), *Distocercospora* (1), *Passalora* (143), *Prathigada* (8), *Pseudocercospora* (527), *Scolecostigmina* (12), *Sirosporium* (15), *Stenellopsis* (1), *Stigmina, Verrucisporota* (4), and *Zasmidium* (88). A staggering 129 new combinations are made, and seven replacement names introduced. It should be noted that in the case of the new names and some combinations, "Kamal" is omitted (corrected by an erratum slip pasted in at the front of the book). Also, where new names are introduced, the authors of the replaced name should not have been placed in parentheses — for example it should be *C. holarrhenigena* "Kamal, nom. nov." and not "(R.K. Srivast., N, Srrivast. & A.K. Srivast.) nom. nov." (p. 51).

In the arrangement of the entries within the accepted genera, the species names precede the generic names, an unusual practice following Crous & Braun (2003). However, here they are not treated as a single alphabetical series but have been separated into the accepted genera. The core elements of the nomenclatural information, including types, also seems to have been copied directly from the 2003 work, with an identical layout and punctuation — and the author does indicate in the introduction that the work is "based largely on Crous and Braun (2003)" (p. 5). However, it is stated that type material in the principle Indian herbaria was examined unless otherwise stated, where decisions were based on descriptions and illustrations. References to hosts and localities in India, in some cases Indian specimens, Indian literature reports, and in some instances notes on the species are added. There are detailed descriptions and line drawings of the newly described species, while other species are illustrated to show "various morphotypes in a single genus" (p. 6).

It would have been helpful to provide potential users keys at least to the genera, although the host list provided may facilitate identification. However,

with no diagnostic information included (apart from the host plants) in the species entries, it will be necessary for users to consult original accounts for details necessary to confirm whether they have that taxon or another on the same host. Nevertheless, this book represents an incredible amount of dedicated work and provides the necessary basis for future revisionary studies using molecular phylogenetic methods — something necessary to resolve unequivocally species circumscriptions and the extent of host specificities in the cercosporoid fungi.'

Chupp C. 1954. A monograph of the fungus genus *Cercospora*. Ithaca, NY: C. Chupp.

Crous PW, Braun U. 2003. *Mycosphaerella* and its anamorphs. 1. Names published in *Cercospora* and *Passalora*. Utrecht: Centraalbureau voor Schimmelcultures.

Vasudeva RS. 1963. Indian *Cercosporae*. New Delhi: Indian Council of Agricultural Research.

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BASIDIOMYCETES

***Strophariaceae* s.l.** By M.E. Noordeloos, 2011. FUNGI EUROPAEI 13. Edizioni Candusso, Via Ottone Primo 90, 17021 Alassio SV, Italy. <maxcandusso@libero.it>. ISBN 978-88-905310-0-2. Pp. 648, pl. 377, figs 43. Price 69.00 €.

The *Strophariaceae* is one of the families in the *Agaricales* whose concept has undergone huge changes — thanks to insights derived from DNA-based phylogenetic analyses. In the morphological sense, it consisted of the brown-spored *Pholiota* and the purple-grey dark-spored genera *Stropharia*, *Psilocybe*, and *Hypholoma*. According to Matheny et al.'s (2007) pivotal work, the family forms one clade with the *Hymenogastraceae* and together they harbour, in addition to the traditional genera, *Hebeloma*, *Galerina*, *Agrocybe*, *Phaeocollybia*, and *Alnicola* (*Naucoria*).

Contrary to what is suggested by the book title, the book deals with the family as originally described and excluding the modern additional genera: in other words, *Strophariaceae* p.p. would have been a better title. However, as changes in the genus concepts as depicted in recently published phylogenies (e.g. Matheny et al. 2007) are accepted, the following genera are included: *Deconica* (including *Melanotus*), *Flammula*, *Hemistropharia*, *Hypholoma*, *Kuehneromyces*, *Leratiomyces*, *Meottomyces*, *Phaeonematoloma*, *Pholiota*, *Psilocybe*, and *Stropharia*. This is a very welcome departure from the broad

genus concept as given by the same author in his floristic treatment of this group for the Dutch agaric flora (Noordeloos 1999). For *Pholiota,* the recent European monograph by Holec (2001) is used as a template.

This book follows the general lay-out of the series, with an introduction providing a history of the family and now current concepts and ideas, a short introduction to the characters that are of value for the identification of the species, and the taxonomic part that gives keys to the genera and species, species descriptions, black-and-white drawings of microscopical characters, a list of references, and almost 200 pages of colour photos —mainly of the fruitbodies in their natural habitat, but also with a number of spore photos and reproductions of important historical illustrations.

The book is bilingual throughout, with the original English text translated into Italian.

The strength of this publication lies in the combination of descriptions and illustrations; especially priceless is that multiple photos are given for many species.

The keys are not easy to use, mainly because the differences between the species are often subtle and difficult to put into words. I find it very frustrating that the differences between *Stropharia* and *Leratiomyces* are not more clear-cut, and I keep wondering whether acanthocytes are really restricted to the genus *Stropharia*. As with any publication of this size, it is easy to find mistakes that could have been caught during the publication process.

This book deserves to find a wide use, and not only in Europe. The price is very reasonable for the amount of information this volume holds.

Holec J. 2001. The genus *Pholiota* in central and western Europe. Libri botanici 20.

Matheny PB, Curtis JM, Hofstetter V, Aime MC, Moncalvo J-M, Ge Z-W, Yang Z-L, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Daniele GM, Desjardin DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS. 2007 ["2006"] Major clades of *Agaricales*: a multilocus phylogenetic overview. Mycologia 98: 982–995.

Noordeloos ME. 1999. *Strophariaceae*. In Bas et al. (editors) Flora agaricina neerlandica 4: 27–106. A.A. Balkema, Rotterdam.

Revision of Malaysian species of *Boletales* s.l. (*Basidiomycota*) described by E.J.H. Corner (1972, 1974). By E. Horak, 2011. [Malaysian Forest Records 51]. Forest Research Institute Malaysia, 52109 Kepong, Selangor Darul Ehsan, Malaysia. <FRIM_Publications@frim.gov.my>. Pp. 245, figs. 127. US$ 53.

The 1972 publication of E.J.H. Corner's *Boletus* in Malaysia (and a 1974 follow-up publication) represents a landmark in the taxonomy of boletoid

fungi. The importance of this work is twofold. First, it provides the first (and still largely unsurpassed) extensive exposition of the prodigious biodiversity of boletes in tropical East Asia. Corner (1972) described nearly 100 new bolete species; furthermore, he noted that many species could not be described due to the sheer abundance of collections and environmental conditions that caused many to decompose before they could be examined, as well as to the abundance of suitable habitat, stating in regard to the latter: "When I consider the immense number of places unexplored mycologically in Malaysia, I think 300 species of boleti may be no exaggeration." Second, Corner's book called into question the tidiness of generic concepts that were constructed around the comparatively depauperate north temperate mycota. Corner maintained that the defining characteristics of many bolete genera (such as color of the spore deposit) did not hold when tested by the tropical mycota, but rather exhibited a continuum between genera.

Despite the importance of this work, its impact on —and utility for— subsequent taxonomic studies in tropical Asia and Australasia has been somewhat diminished by taxonomic out-datedness and the restricted availability of type collections and other material examined by Corner. In accordance with his observations on intergradation of characters between genera, Corner (1972) recognized only four bolete genera —*Boletus*, *Gyroporus*, *Heimiella* (now *Heimioporus*), and *Strobilomyces*— while placing many taxa under subgenera (e.g., *Austroboletus*, *Leccinum*, *Tylopilus*) that are widely recognized at generic rank by other workers. Therefore, many taxa are in need of nomenclatural revision to bring them up to date with current taxonomic concepts. As a result of high humidity in both field and herbarium storage localities, primitive field conditions for drying specimens, and storage methods, many of Corner's collections were damaged by molds, degraded by storage in alcohol formalin (or hardened by evaporation of AF during storage), or rendered toxic by treatment with mercuric chloride; restricted access to collections and restricted accessibility of important microcharacters have been the result.

Given the importance of Corner's collections and the difficulties inherent in their study, the present revision by Egon Horak is a most welcome and important contribution to the taxonomic study of boletes. Dr. Horak presents revised descriptions of taxa combining information from Corner's original protologues and Latin diagnoses (the latter translated into English to facilitate study), data gained from additional studies of Corner's holotypes and other collections, and data from studies of holotype collections from other collectors (including Baker, Heim, Höhnel, Hooker, and Ridley, among others) for those species included but not described by Corner. Horak provides analyses of 160 (124 accepted, 22 doubtful, and 14 rejected, excluded, or invalid) species, including emended descriptions, line drawings of microscopic features (127 figures),

lists of observations and conclusions from his studies, and nomenclatural changes necessary for bringing the nomenclature of Corner's taxa up-to-date with current concepts. Additional features include taxonomic keys to all of the treated taxa, a list of additional important taxonomic studies of boletes from the Far East, a synopsis of all of the treated taxa arranged by currently accepted genus, and species list indexed by specific epithet. Perhaps most importantly, Horak brings to this study his own extensive personal experience working in Malaysia and the Asian tropics, and is therefore able to conceptualize Corner's taxa in a broad comparative context.

Any taxonomic revision requires some judgments as to the placement of species in more inclusive taxa. In the boletes, a longstanding taxonomic issue involves character weighting of species with pale short-elliptical or phaseoliform basidiospores; an example of this issue is *Rubinoboletus*, in which species have been placed that share this spore morphology but differ significantly in other characteristics (see Osmundson and Halling 2010). In the present revision, Horak places a number of species with pale phaseoliform basidiospores in *Gyroporus*, and in so doing emphasizes spore morphology over characteristics (e.g., presence/absence of clamp connections, orientation of hyphae in the stipe) emphasized by some previous authors. In the case of *Gyroporus balloui* (Peck) E. Horak (= *Tylopilus balloui* (Peck) Singer), this placement is contradicted by molecular evidence presented after the preparation of Dr. Horak's volume (Osmundson and Halling 2010), but other species remain to be evaluated in the light of DNA sequence data. In the meantime, a provisional placement based on spore morphology seems reasonable; this example emphasizes that some of our present taxonomic concepts contain inherent contradictions and may very well change in the light of additional data and analyses.

In *Boletus* in Malaysia, Corner – considering existing taxonomic problems in the boletes – wrote, "I have no doubt that if the fungus flora of Malaysia can be explored before the destruction of the main forests or the elimination of the non-commercial trees, fresh discoveries will resolve these problems." Biodiversity discovery, conservation, and the solution to taxonomic problems share the need for carefully researched scholarly work on the description and revision of species and higher taxa. Horak states that "the present revision is only one further step in the direction to unravel the numerous bolete taxa described from SE-Asia and Australasia;" however, his "one further step" is a critical one: by providing a detailed re-examination of Corner's specimens and by bringing the taxonomy of Corner's taxa into a modern context, Horak has made two highly valuable contributions to the study of boletes. In this outstanding volume, Horak will not only earn the gratitude of boletologists, but has provided an important tool for assessing diversity, rarity, endemicity and —unfortunately, but quite likely, given the extent and rate of forest conversion

in Malaysia— extinction of the morphologically striking and ecologically important bolete fungi of the East Asian tropics.

Corner EJH. 1972. *Boletus* in Malaysia. Government Printing Office, Singapore. 263 pp.

Corner EJH. 1974. *Boletus* and *Phylloporus* in Malaysia: further notes and descriptions. Garden's Bull. Singapore 27:1-16.

Osmundson TW, Halling RE. 2010. *Tylopilus oradivensis* sp. nov.: a newly described member of the *Tylopilus balloui* complex from Costa Rica. Mycotaxon 113: 475–483. http://dx.doi.org/10.5248/113.475

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MYCOFLORISTICS

A guidebook to the macrofungi of Fraser's Hill. By B.K. Thi, S.S. Lee, N. Zanuddin & H.T. Chan, 2011. SIRI ALAM DAN RIMBA NO. 14. Forest Research Institute Malaysia, 52109 Kepong, Sealngor Darul Ehsan, Malaysia, <FRIM_Publications@frim.gov.my>. ISBN 978-967-5221-63-7. Pp xi + 93, plates. Price US$ 23.

This little guide starts with an introduction to Fraser's Hill, situated in the state of Pahang, Malaysia, around 100 km from the capital, Kuala Lumpur. Fraser's Hill is not only a draw for tourists, it is also well known among mycologists (see e.g. Tan et al. 2009). The mountainous terrain is accessible along some well-maintained trails and a road. The world of the fungi is also introduced to the readers, after which the main part follows with photos and short descriptions and notes on the most colourful and attractive mushroom-forming fungi of all taxonomic groups. It is a pleasure to see that the showy purple floccose *Lepiota*-like species, recently placed in the new genus *Coniolepiota* (Vellinga et al. 2011), is also present in this part of Malaysia, and that another *Lepiota*-like taxon in the *L. furfuraceipes* group (described from China) extends into Malaysia as well. A nice collection of boletes is illustrated. We hope that this book will be widely available at the park, opening the eyes of the visitors to the exciting world of fungi. And that it will serve as an example for other forests in Malaysia and beyond.

Tan Y-S, Desjardin DE, Perry BA, Vikineswary S, Noorlidah A. 2009. *Marasmius* sensu strico in Peninsular Malaysia. Fungal Diversity 37: 9–100.

Vellinga EC, Sysouphanthong P, Hyde KD. 2011. The family *Agaricaceae*: phylogenies and two new white-spored genera. Mycologia 103: 494–509. http://dx.doi.org/10.3852/10-204

Los hongos de Panamá. Introducción a la identificación de los macroscópicos. By G. Guzmán & M. Piepenbring, 2011. Pp. 386. pl. 798. Price US$ 45.

Panama is a well-known biodiversity hotspot, and yet, little is known about its fungal diversity. Recently a preliminary checklist of fungi was published (Piepenbring 2006) in which around 1800 taxa are listed. A new, richly illustrated book of this Central American state shows the diversity of the macrofungi and demonstrates that our knowledge of the fungi is still insufficient with many species still to be undescribed.

The book is divided into four parts: an introduction to the country, its habitats, and the fungi; the classification of the fungi; a key to the species treated in the book; and lastly, but most importantly, the species are introduced. They are treated alphabetically with colour photos provided for each species and with line drawings of microscopical details also present. A glossary, index, and a list of the species in a taxonomic framework complete the work.

A bit unusual, but welcome, for a book like this is that lichens are also included as well as the slime molds.

The illustrations are often quite large, covering a complete page. In many cases, several photos are given for one species, showing the mushroom from different angles or in different stages.

It is really interesting to see that some 'temperate' species —such as *Cortinarius violaceus*— occur in Panama. But the highlight of a book like this is that it introduces non-tropical readers to the unfamiliar forms and shapes of tropical fungi such as *Staheliomyces cinctus* and *Poronia oedipus*.

The genus names are in many cases not quite up to date (e.g. *Collybia* for what now is called *Gymnopus*; *Coprinus* instead of *Coprinopsis*); photos are not always sharp, and colours seem to be a little off in several cases.

But the biggest asset of a book like this is that it shows clearly that the times of fungal discovery and taxonomy are far from over.

Piepenbring, M. 2006. Checklist of fungi in Panama. Preliminary version. Puente Biológica 1: 1-190.

Book Announcement

***Ustilaginales* of India.** By R.V. Gandhe, 2011. Bishen Singh Mahendra Pal Singh, 23-A, New Connaught Place, Dehra Dun, 248001 India, <bsmps@vsnl.com>. ISBN 978-81-211-0788-4. 414 pp. ill. circa US$ 154.00

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Regional annotated mycobiotas new to www.mycotaxon.com

Mycotaxon is pleased to announce the addition of a new url link on our "web-list" page at www.mycotaxon.com//resources/weblists.html, which will connect to the checklist PDF now available for free download from the posted off-site webpage. The content and design of each list is the sole responsibility of its authors and their three (or more) expert reviewers, after which those meeting Mycotaxon's scientific and nomenclatural criteria are accepted for posting. The author, title, and abstract of our most recently accepted web-list is provided below.

EURASIA AND THE MID-EAST

Armenia, Azerbaijan, Georgia, Iran, Russia, Turkey

Ghobad-Nejhad, Masoomeh. Updated checklist of corticioid and poroid basidiomycetes of the Caucasus region. 72 pp.

Abstract — A first annotated checklist of corticioid and poroid basidiomycetes of the Caucasus region was published in 2009 (Ghobad-Nejhad et al., Mycologia Balcanica 6: 123–168, 2009). A new, updated version of the checklist is presented and is aimed to be regularly updated at an Iranian mycological website. The list includes 621 accepted species names with their synonym(s) used in the bibliographic literature. Major updates include: 1) addition of substratum data for the species based on numerous specimens examined from different herbaria, 2) addition of literature references, distribution data and species names not present in the earlier version, and deletion of names confirmed not to occur in the region, and 3) revision of nomenclature and synonyms. Compared to the 2009 list, 86% of species in the current checklist are supported by specimens examined. The aim to post the checklist in an online version is to provide a permanent repository for keeping the list updated with no unnecessary delay and for making the data available to the mycologists interested in fungal diversity of the Caucasus biodiversity hotspot. New records for the region will be published online and the users will be able to learn about the most recent changes to the checklist via subscription to a feed (RSS). The link to the checklist is: http://www.myco-lich.com/mycology-of-iran/basidiomycota/caucasus-region/checklists

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Fungal nomenclature.

Changes to publication requirements made at the XVIII International Botanical Congress in Melbourne — what does e-publication mean for you?

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Abstract — Changes to the International Code of Botanical Nomenclature are decided on every six years at Nomenclature Sections associated with International Botanical Congresses (IBC). The XVIII IBC was held in Melbourne, Australia; the Nomenclature Section met from 18-22 July 2011 and its decisions were accepted by the Congress at its plenary session on 30 July. Several important changes were made to the Code as a result of this meeting that will affect publication of new names. Two of these changes will come into effect on 1 January 2012, some months before the Melbourne Code is published. Electronic material published online in Portable Document Format (PDF) with an International Standard Serial Number (ISSN) or an International Standard Book Number (ISBN) will constitute effective publication, and the requirement for a Latin description or diagnosis for names of new taxa will be changed to a requirement for a description or diagnosis in either Latin or English. In addition, effective from 1 January 2013, new names of organisms treated as fungi must, in order to be validly published, include in the protologue (everything associated with a name at its valid publication) the citation of an identifier issued by a recognized repository (such as MycoBank). Draft text of the new articles dealing with electronic publication is provided and best practice is outlined.

To encourage dissemination of the changes made to the International Code of Nomenclature for algae, fungi, and plants, this article will be published in Botanical Journal of the Linnean Society, BMC Evolutionary Biology, Cladistics, Mycotaxon, MycoKeys, New Phytologist, North American Fungi, Novon, Opuscula Philolichenum, PhytoKeys, Phytoneuron, Phytotaxa, PLoS ONE, Systematic Botany, and Taxon.

Introduction

At the XVIII International Botanical Congress in Melbourne, Australia, in July 2011, two important changes were made to the International

Code of Botanical Nomenclature (now the International Code of Nomenclature for algae, fungi, and plants) that will take effect from 1 January 2012. These changes will affect everyone who publishes names governed by this Code. As the Melbourne Code will not be published until approximately mid-2012, we felt it would be helpful to outline these changes, particularly those concerning effective publication in electronic media (in Articles 29, 30, and 31). For a concise report on all the changes to the Code accepted in Melbourne, see McNeill & al. (2011).

A draft wording of the revised Articles, Notes, and Recommendations on effective publication is provided to aid editors and publishers in establishing best practice for implementing this aspect of the Code. We also outline here what these changes do not mean, in order to guide those wishing to publish new names and typifications by electronic means. We urge readers to consult the report of the Special Committee on Electronic Publication accompanying the changes proposed prior to the Congress (Chapman & al. 2010), wherein the reasoning for the changes now accepted into the Code is set out.

Draft wording of revised Articles 29, 30, and 31 and Recommendations 29A, 30A, and 31A

Here we reproduce the wording of all of the relevant Articles, Notes, and Recommendations (omitting the Examples), with the changes highlighted in **bold**. The wording here is provisional, pending the meeting of the Editorial Committee in December 2011 to finalize the printed version of the Melbourne Code.

Article 29

29.1. Publication is effected, under this Code, by distribution of printed matter (through sale, exchange or gift) to the general public or at least to botanical institutions with libraries accessible to botanists generally. **Publication is also effected by electronic distribution of material in Portable Document Format (PDF; see also Art. 29.3 and Rec. 29A.1) in an online publication with an International Standard Serial Number (ISSN) or an International Standard Book Number (ISBN).** Publication is not effected by communication of new names at a public meeting, by the placing of names in collections or gardens open to the public, by the issue of microfilm made from manuscripts, typescripts or other unpublished material, or by distribution electronically **other than as described above**.

29.2. **For the purpose of this Article, "online" is defined as accessible electronically via the World Wide Web.**

29.3. **Should Portable Document Format (PDF) be succeeded, a successor international standard format communicated by the General Committee (see Div. III) is acceptable.**

29.4. **The content of a particular electronic publication must not be altered after it is first issued. Any such alterations are not themselves effectively published. Corrections or revisions must be issued separately to be effectively published.**

Recommendation 29A

[Existing Recommendation replaced by the following]

29A.1. **Publication electronically in Portable Document Format (PDF) should comply with the PDF/A archival standard (ISO 19005).**

29A.2. **Authors should preferably publish in publications that are archived, satisfying the following criteria as far as is practical (see also Rec. 29A.1):**

(a) **The material should be placed in multiple trusted online digital repositories, e.g. an ISO-certified repository;**

(b) **Digital repositories should be in more than one area of the world and preferably on different continents;**

(c) **Deposition of printed copies in libraries in more than one area of the world and preferably on different continents is also advisable.**

Article 30

30.1. **Publication by distribution of electronic material does not constitute effective publication before 1 January 2012.**

30.2. **An electronic publication is not effectively published if there is evidence associated with or within the publication that it is merely a preliminary version that was, or is to be, replaced by a version that the publisher considers final, in which case only that final version is effectively published.**

30.3. Publication by indelible autograph before 1 January 1953 is effective. Indelible autograph produced at a later date is not effectively published.

30.4. For the purpose of this Article, indelible autograph is handwritten material reproduced by some mechanical or graphic process (such as lithography, offset, or metallic etching).

30.5. Publication on or after 1 January 1953 in trade catalogues or non-scientific newspapers, and on or after 1 January 1973 in seed-exchange lists, does not constitute effective publication.

30.6. The distribution on or after 1 January 1953 of printed matter accompanying exsiccatae does not constitute effective publication.

Note 1. If the printed matter is also distributed independently of the exsiccata, it is effectively published.

30.7. Publication on or after 1 January 1953 of an independent non-serial work stated to be a thesis submitted to a university or other institute of education for the purpose of obtaining a degree is not effectively published unless it includes an explicit statement (referring to the requirements of the Code for effective publication) or other internal evidence that it is regarded as an effective publication by its author or publisher.

Note 2. The presence of an International Standard Book Number (ISBN) or a statement of the name of the printer, publisher, or distributor in the original printed version is regarded as internal evidence that the work was intended to be effectively published.

Recommendation 30A

30A.1. **Preliminary and final versions of the same electronic publication should be clearly indicated as such when they are first issued.**

30A.2. It is strongly recommended that authors avoid publishing new names and descriptions or diagnoses of new taxa (nomenclatural novelties) in ephemeral printed

matter of any kind, in particular printed matter that is multiplied in restricted and uncertain numbers, in which the permanence of the text may be limited, for which effective publication in terms of number of copies is not obvious, or that is unlikely to reach the general public. Authors should also avoid publishing new names and descriptions or diagnoses in popular periodicals, in abstracting journals, or on correction slips.

30A.3. To aid availability through time and place, authors publishing nomenclatural novelties should give preference to periodicals that regularly publish taxonomic articles. **Otherwise, a copy of a publication (whether published as printed or electronic matter) should be sent to an indexing centre appropriate to the taxonomic group, and publications that exist only as printed matter** should be deposited in at least ten, but preferably more, botanical or other generally accessible libraries throughout the world.

30A.4. Authors and editors are encouraged to mention nomenclatural novelties in the summary or abstract, or list them in an index in the publication.

Article 31

31.1. The date of effective publication is the date on which the printed **or electronic** matter became available as defined in Art. 29 and 30. In the absence of proof establishing some other date, the one appearing in the printed **or electronic** matter must be accepted as correct.

[Existing Note 1 replaced by the following]

***31.2.* When a publication is issued in parallel electronic and printed versions, these must be treated as effectively published on the same date unless the dates of the versions are different according to Art. 31.1.**

31.3. When separates from periodicals or other works placed on sale are issued in advance, the date on the separate is accepted as the date of effective publication unless there is evidence that it is erroneous.

Recommendation 31A

31A.1. The date on which the publisher or publisher's agent delivers printed matter to one of the usual carriers for distribution to the public should be accepted as its date of effective publication.

Best practice

Authors of new names, editors and publishers will all be interested in ensuring that the publications including new names are in accordance with the Melbourne Code, so that the names therein are effectively published. We suggest that those publishing in journals or monograph series and books that have online editions communicate with the editors so that best practice can be established across the community as quickly as possible. Many publishers have been carefully addressing the issues involved with the e-publication of novelties for some time (see Knapp & Wright 2010; guidelines in PLoS ONE, http://www.plosone.org/static/policies.action#taxon) and considerable interest in making these new Code changes function effectively has been apparent.

Some practices that we feel will help with the initial stages of e-publication of novelties that are according to the Melbourne Code are:

- Having each article bear the date of publication prominently (as is done in many journals, for example New Phytologist or Nature).
- If an online early version is issued that is not the same as the final version (and thus not the place of effective publication) stamp each article with this fact prominently (for example American Journal of Botany).
- Prominent display of the ISSN or ISBN of the publication on each article will help indexers establish effective publication.
- Publication in journals (or monograph series) that participate in the CLOCKSS system (see Knapp & Wright, 2010, for a description) or another international archive and preservation system will ensure long-term archiving.
- Authors of new names by electronic means should alert the appropriate indexing center as recommended in Rec. 30.A.3 – this will help indexers who may otherwise not be aware of electronically published names.

What these changes do not mean

Although the new Articles and Recommendations use the terms PDF and PDF/A, this does not mean that publications must be issued *only* in that format to be effectively published. For example, some online journals issue papers in Hypertext Markup Language (HTML) format together with a parallel PDF version. In such cases, the PDF version will be effectively published. The stipulation that the General Committee for Botanical Nomenclature will communicate the acceptability of a new international standard format, should PDF ever be succeeded, means authors of novelties and the community using the Code can remain informed as to advances in the field and that the Code will be protected from obsolescence.

Use of the following means of electronic publication will NOT result in effective publication of novelties under the Melbourne Code.

- Publication on websites or in ephemeral documents available over the Internet (there are strict criteria for granting of ISSNs — see http://www.issn.org/).
- Publication in journals without a registered ISSN or e-ISSN.
- Publication in books without a registered ISBN or e-ISBN.

The Recommendation approved to advise the deposition of a hard copy of any e-publication in a library suggests to botanists an action, but it does not set out standard practice or a protocol for librarians to follow. Librarians are themselves in a complex transition zone between publication modalities (Johnson & Luther

2007), and botanists may find them unwilling or unable to accommodate single hard copy papers as individual accessions should the volume be great.

Two other important changes to the Code relating to the publication of names

The second change to the Code approved in Melbourne to take effect from 1 January 2012 is that the description or diagnosis required for valid publication of the name of a new taxon of all organisms falling under the Code may be in either English or Latin. This is the current provision for names of plant fossils, but all new non-fossil taxa have required a Latin description or diagnosis (fungi and plants from 1 January 1935; algae [including cyanobacteria, if treated under the Code] from 1 January 1958). This has no bearing on the form of scientific names, which continue to be Latin or treated as Latin. Individual journal requirements for Latin and/or English will, of course, be determined by the editors of those journals.

A third change to the Code approved in Melbourne relating to publication of names, but one not taking effect until 1 January 2013 (not 1 January 2012 as reported by Miller & al., 2011), is that all new names of organisms treated as fungi must, as an additional requirement for valid publication, include in the protologue the citation of an identifier issued by a recognized repository (such as MycoBank, http://www.mycobank.org/). This will be publicized separately.

The requirement for a unique identifier for new names of fungi on or after 1 January 2013 does not apply to plants or algae; there is no need for authors of new names in these groups to request LSIDs (Life Science Identifiers) – or other identifiers – from indexing centers.

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To encourage dissemination of the changes made to the International Code of Nomenclature for algae, fungi, and plants, this article will be published in Botanical Journal of the Linnean Society, BMC Evolutionary Biology, Cladistics, Mycotaxon, MycoKeys, New Phytologist, North American Fungi, Novon, Opuscula Philolichenum, PhytoKeys, Phytoneuron, Phytotaxa, PLoS ONE, Systematic Botany, and Taxon.

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Volume 117, pp. 517–519 *July–September 2011*

Nomenclatural novelties and typifications proposed in Mycotaxon 117

Albahypha Oehl, G.A. Silva, B.T. Goto & Sieverd., p. 308

Albahypha drummondii (Błaszk. & Renker) Sieverd., Oehl, B.T. Goto & G.A. Silva, p. 308

Albahypha walkeri (Błaszk. & Renker) Sieverd., Oehl, B.T. Goto & G.A. Silva, p. 309

Amanita conara Tulloss & Halling, p. 180

Amanita costaricensis Tulloss, Halling, G.M. Muell. & Singer, p. 189

Amanita garabitoana Tulloss, Halling & G.M. Muell., p. 169

Amanita vernicoccora Bojantchev & R.M. Davis, p. 491

Ambispora nicolsonii (C. Walker, L.E. Reed & F.E. Sanders) Oehl, G.A. Silva, B.T. Goto & Sieverd., p. 431

Anaselenosporella indica Pratibha, Bhat & Raghuk., p. 269

Arachnophora goanensis Pratibha, Bhat & Raghuk., p. 272

Archaeospora myriocarpa (Spain, Sieverd. & N.C. Schenck) Oehl, G.A. Silva, B.T. Goto & Sieverd., p. 430

Archaeospora undulata (Sieverd.) Sieverd., G.A. Silva, B.T. Goto & Oehl, p. 430

Arthrobotrys latispora H.Y. Su & X.Y. Yang, p. 32

Caloplaca allochroa Y. Joshi, Vondrák & Hur, p. 263

Cephalotrichum acutisporum J.J. Xu & T.Y. Zhang, p. 208

Cephalotrichum castaneum (Y.L. Jiang & T.Y. Zhang) Y.L. Jiang & T.Y. Zhang, p. 224

Cephalotrichum cylindrosporum Y.L. Zhang & T.Y. Zhang, p. 209

Cephalotrichum ellipsoideum H.Q. Pan & T.Y. Zhang, p. 211

Cephalotrichum inflatum Y.L. Jiang & T.Y. Zhang, p. 213

Cephalotrichum longicollum Y.L. Jiang & T.Y. Zhang, p. 213

Cephalotrichum macrosporum Y.L. Jiang & T.Y. Zhang, p. 214

Cephalotrichum oblongum J.J. Xu & T.Y. Zhang, p. 216

Cephalotrichum ovoideum Y.L. Jiang & T.Y. Zhang, p. 217

Cephalotrichum robustum Y.L. Jiang & T.Y. Zhang, p. 218

Cephalotrichum spirale H.M. Liu, H.Q. Pan & T.Y. Zhang, p. 220

Cephalotrichum terricola Y.L. Jiang & T.Y. Zhang, p. 221
Cephalotrichum verrucipes Y.L. Jiang & T.Y. Zhang, p. 223
Cephalotrichum verrucisporum (Y.L. Jiang & T.Y. Zhang) Y.L. Jiang & T.Y. Zhang, p. 224
Ceratocystis mangicola M. van Wyk & M.J. Wingf., p. 395
Ceratocystis mangivora M. van Wyk & M.J. Wingf., p. 397
Cetraspora nodosa (Błaszk.) Oehl, G.A. Silva, B.T. Goto & Sieverd., p. 431
Cortinarius callimorphus Bojantchev & R.M. Davis, p. 3
Corynespora fujianensis L.G. Ma & X.G. Zhang, p. 355
Craspedodidymum fujianense L.G. Ma & X.G. Zhang, p. 351
Daldinia grayana Lar.N. Vassiljeva & S.L. Stephenson, p. 332
Dendrothele latenavicularis Gorjón, p. 102
Diatrypella informis Ellis & Everh. ex Lar.N. Vassiljeva & S.L. Stephenson, p. 335
Ellisembia schimae Jian Ma & X.G. Zhang, p. 247
Endophragmiella clausenae L.G. Ma & X.G. Zhang, p. 280
Endophragmiella nanlingensis S.C. Ren & X.G. Zhang, p. 123
Endophragmiella pentaphylacis L.G. Ma & X.G. Zhang, p. 281
Gymnopus fuscotramus Mešić, Tkalčec & Chun Y. Deng, p. 324
Junghuhnia minor H.S. Yuan, p. 256
Lepiota aureofulvella Sysouphanthong, K.D. Hyde, Chukeatirote & Vellinga, p. 62
Lepiota pallidiochracea J.F. Liang & Zhu L. Yang, p. 360
Lepiota papillata Sysouphanthong, K.D. Hyde, Chukeatirote & Vellinga, p. 75
Lignincola conchicola J.K. Liu, E.B.G. Jones & K.D. Hyde, p. 344
Linkosia hibisci Jian Ma & X.G. Zhang, p. 249
Macrolepiota rhodosperma var. *velicopia* Vizzini & Contu, p. 153
Macrolepiota procera f. *fuliginosa* (Barla) Vizzini & Contu, p. 158
(lectotypified, epitypified)
Macrolepiota procera f. *permixta* (Barla) Vizzini & Contu, p. 161
(epitypified)
Minimelanolocus linderae Jian Ma & X.G. Zhang, p. 131
Minimelanolocus mori Jian Ma & X.G. Zhang, p. 133
Moelleriella pumatensis T.T. Nguyen & N.L. Tran, p. 48
Mycena lomavritha Manim., p. 244
≡ *Mycena indica* Manim. & Leelav. 1989 ("1988"),
non *Mycena indica* Sarwal & Rawla 1983
Mycena saloma Aravind. & Manim., p. 240
Nemania pseudoillita (Lar.N. Vassiljeva) Lar.N. Vassiljeva & S.L. Stephenson, p. 339
Passalora barringtoniigena R. Singh, Sham. Kumar & Kamal, p. 138
Passalora wangii (F.Y. Zhai, Y.L. Guo & Yu Li) F.Y. Zhai, Y.L. Guo & Yu Li, p. 365
Phylloporus fagicola Montoya & Bandala, p. 10

Pouzarella albostrigosa Largent & Abell-Davis, p. 470
Pouzarella debilis (Corner & E. Horak) Largent & Abell-Davis, p. 463
Pouzarella farinosa Largent & Skye Moore, p. 455
Pouzarella fusca Largent & Abell-Davis, p. 466
Pouzarella lageniformis Largent & Skye Moore, p. 443
*Pouzarella lasia (*Berk. & Broome) Largent & Abell-Davis, p. 474
Pouzarella pamiae Largent, p. 450
Pouzarella parvula Largent & Skye Moore, p. 447
Pouzarella pilocystidiata Largent & Skye Moore, p. 439
Pouzarella setiformis Largent & Abell-Davis, p. 459
Pseudocercospora kamalii Rajeshkumar, Rahul Sharma & S.K. Singh p. 232
Pseudocercospora miliusae R. Singh, Sham. Kumar & Kamal, p. 140
Rhexodenticula zhengii D.W. Li & Jing Y. Chen, p. 288
Sacculospora Oehl, Sieverd., G.A. Silva, B.T. Goto, I.C. Sánchez & Palenz., p. 311
Sacculospora baltica (Błaszk., Madej & Tadych) Oehl, Palenz., I.C. Sánchez, B.T. Goto, G.A. Silva & Sieverd, p. 311
Sacculosporaceae Oehl, Sieverd., G.A. Silva, B.T. Goto, I.C. Sánchez & Palenz., p. 310
Septobasidium atalantiae S.Z. Chen & L. Guo, p. 291
Stanjehughesia micheliae Jian Ma & X.G. Zhang, p. 251
Terriera huangshanensis Z.Z. Yang, Y.R. Lin & C.L. Hou, p. 368
Tricispora Oehl, Sieverd., G.A. Silva & Palenz., p. 310
Tricispora nevadensis (Palenz., N. Ferrol, Azcón-Aguilar & Oehl) Oehl, Palenz., G.A. Silva & Sieverd., p. 310

bad taxonomy

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